RESOURCE LIMITATION OF AUTOTROPHS AND HETEROTROPHS IN BOREAL FOREST HEADWATER STREAMS

By

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Abstract

In stream biofilms, autotrophs and heterotrophs are responsible for the majority of in-stream nutrient transformations. In boreal forest catchments, discontinuous permafrost can lead to variation in nutrient and energy resources, which can control competition for nutrients between autotrophs and heterotrophs within these biofilms. I was interested in determining what resources control nutrient utilization by autotrophs and heterotrophs in headwater streams in the boreal forest of interior Alaska. I hypothesized that the outcome of competition between autotrophs and heterotrophs for inorganic nutrients would be dependent on the availability of (i) organic carbon, (ii) light, or (iii) inorganic nutrients. To measure resource limitation and competition at both patch and reach scales, I deployed nutrient diffusing substrata and conducted nutrient uptake experiments in streams along a permafrost gradient at the Caribou-Poker Creeks Research Watershed in interior Alaska. At the patch scale, autotrophs were light and nutrient limited, whereas heterotrophs were carbon and nutrient limited, and at the reach scale, light had the largest influence on nutrient uptake. Heterotrophs exhibited a larger response to nutrient enrichment when stream ambient carbon stocks were more bioavailable. Autotrophic biomass and productivity was suppressed when labile carbon was available to heterotrophs, suggesting that heterotrophs outcompete autotrophs for nutrients when a labile carbon source is introduced. The positive responses to nutrient and carbon additions suggest that the hypothesized increased nutrient and carbon exports into fluvial networks with permafrost degradation will impact biofilm structure and function, with the potential to influence nutrient export and stream ecosystem function downstream.

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Preface

This thesis was prepared in manuscript format. Chapter 1 is a general introduction outlining the biogeochemistry and nutrient limitation of boreal forest stream biofilms and how these stream ecosystems may be altered by a changing climate. Chapter 2, titled "Resource limitation of autotrophs and heterotrophs in boreal forest headwater streams," was prepared for submission to *Freshwater Science*. Chapter 3 includes brief general conclusions.

I would like to thank my advisor, Jay Jones, for the opportunity to take part in boreal forest stream ecology research in Alaska and for his direction and mentoring throughout this project. I would also like to acknowledge the members of my committee, Roger Ruess and Mary Beth Leigh, for providing beneficial feedback and for being supportive and encouraging throughout my coursework, proposal development, and thesis preparation. Christina Baker, Sarah Carrigan, Audrey Mutschlecner, Marie Schmidt, and Rachel Voight all provided invaluable assistance in both the field and lab throughout project development and during the 2017 and 2018 field seasons. Finally, I would like to gratefully acknowledge Claire Ruffing and Sara Wilbur for providing essential advice, guidance, and friendship throughout my time as a master's student at UAF.

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Chapter 1: General Introduction

1.1 Resource limitation of stream biofilms

Microbes are responsible for the majority of the biogeochemical cycling and metabolic activity in headwater streams (Besemer et al. 2013, Battin et al. 2016). In fluvial networks, autotrophs (i.e., algae and cyanobacteria) and heterotrophs (i.e., fungi, bacteria, and Archaea) form complex aggregate biofilms (Lock et al. 1984) by colonizing the organic and physical structures along a streambed. Often thought of as the biological boundary between benthic and hyporheic zones (Battin et al. 2007), biofilms dominate the microbial activity in freshwater ecosystems and form hot spots of in-stream metabolic processes. These biogeochemical processes include the transformations and mineralization of essential nutrients (Van Horn et al. 2011), extracellular enzyme production (Romani and Sabater 2001), and decomposition of allochthonous (terrestrially-derived) and autochthonous (algal-derived) organic carbon (Battin et al. 2008). Autotrophs also serve as the base of the stream food web, providing energy to higher trophic levels through photoautotrophic primary production (Risse-Buhl et al. 2012).

The energy and nutrient resources required by benthic primary producers and consumers vary over time and throughout stream networks (Mulholland et al. 2001, Bernot et al. 2010). Carbon abundance and lability can limit heterotrophic respiration (Bernhardt and Likens 2002, Robbins et al. 2017). Heterotrophic microbial consumers can use either organic or inorganic nutrients for nutrient uptake and respiration (Burrows et al. 2015, Myrstener et al. 2018), but prefer inorganic nutrients when available (Cotner and Wetzel 1992). Heterotrophs can assimilate these nutrients directly from the water column or the detrital substrate they colonize. Like heterotrophs, photoautotrophs are sensitive to in-stream inorganic nutrient concentrations for productivity (Rier and Stevenson 2002, Tank and Dodds 2003, Myrstener et al. 2018), but are

additionally reliant on light to fix carbon through photosynthesis (Hill et al. 2009). Both autotrophs and heterotrophs are sensitive to changes in thermal regimes, which can interact with resource availability to impact metabolic rates and nutrient uptake (Rasmussen et al. 2011, Cross et al. 2015, Hood et al. 2018). Therefore, in headwater streams, autotrophs and heterotrophs are competing for inorganic nutrients with the competitive outcome dependent on light availability, dissolved organic matter (DOM) lability, and temperature (Fig. 1.1).

Small changes in nutrient concentrations can have substantial impacts on stream periphyton, and nitrogen and phosphorus often limit productivity and carbon use and utilization in pristine headwater streams (Cross et al. 2005, Dodds and Smith 2016). Because microbes assimilate nutrients at relatively fixed ratios, water column nutrient stoichiometry of dissolved organic carbon, nitrogen, and phosphorus impacts nutrient uptake rates (Schade et al. 2011, Piper et al. 2017), and these essential resources can be co-limiting (Elser et al. 2007). Autotrophs are considered to exhibit greater flexibility in nutrient stoichiometry than heterotrophs; their elemental composition can fluctuate as resource availability (i.e. light and inorganic nutrient concentration) varies (Sterner et al. 1998). Heterotrophs, however, must maintain a more constant nutrient stoichiometry (Persson et al. 2010). They can achieve this stoichiometric homeostasis when nutrient availability is low by releasing extracellular enzymes into the water column (Pastor et al. 2019). Extracellular enzyme upregulation helps break down recalcitrant DOM into less complex forms that can be readily assimilated by both autotrophs and heterotrophs (Mann et al. 2014). Additionally, nutrient supply controls heterotrophic use of labile autochthonous carbon (Lyon and Ziegler 2009, Ziegler et al. 2009), and has been shown to support nutrient and carbon retention and recycling in periphyton when inorganic nitrogen and phosphorus are depleted. These studies highlight an important link between light availability, as

a control of algal-derived carbon, and inorganic nutrient recycling and carbon flow from autotrophs to heterotrophs within biofilms, which has implications for within-biofilm carbon utilization and export further downstream (Battin et al. 2016).

Light availability to stream channels controls autotrophic productivity and nutrient uptake through photosynthesis, which in turn can affect heterotrophic resource use. In higher-order streams with open channels and high light penetration, autotrophic algae and cyanobacteria can dominate metabolism and nutrient uptake through carbon fixation (Dodds 2007); whereas in channels heavily shaded by riparian vegetation, heterotrophic bacteria and fungi dominate metabolism and nutrient uptake, primarily using terrestrially-derived organic matter as a carbon source for respiration (Fisher and Likens 1973, Kaplan et al. 2008). Marcarelli et al. (2009) hypothesized when organic carbon is depleted, and light is readily available, autotrophs fix carbon and therefore outcompete heterotrophs for inorganic nutrients. Conversely, this autochthonous, algal-derived carbon can provide a bioavailable energy source to fuel heterotrophic respiration (Thorp and Delong 2002), and can stimulate heterotrophic decomposition of recalcitrant allochthonous DOM through algal mediated priming (Kuehn et al. 2014, Rier et al. 2014).

1.2 *Discontinuous permafrost and boreal forest stream biogeochemistry*

In the boreal forest, permafrost distribution and extent has a large effect on watershed vegetation stand structure, soil moisture and temperature, hydrology, and biogeochemistry. Permafrost, or perennially frozen ground, covers approximately 24% of the land in the northern hemisphere (Zhang et al. 2008). In interior Alaska, an estimated 80% of the landscape is underlain with discontinuous permafrost, with 34% occurring as near-surface permafrost (Slater

and Lawrence 2013, Pastick et al. 2015). This frozen ground is relatively impermeable and permafrost distribution controls water movement through catchments. In areas underlain by permafrost, watershed flowpaths are largely restricted to the active layer (the surface soil that thaws and refreezes annually). In catchments with little or no permafrost, ground water can infiltrate deeper through unfrozen soil into the mineral layer (Walvoord and Striegl 2007) before flowing to streams. The depth water infiltrates through soil layers affects microbial processing and therefore groundwater water chemistry and runoff entering streams, with implications for stream microbial communities.

Permafrost affects stream biogeochemistry by controlling carbon and nutrient availability to benthic organisms as well as export to downstream ecosystems (Fig. 1.2). In the boreal forest, permafrost is rich in organic matter, storing large amounts of organic carbon (Zimov et al. 2006), but also largely unknown nitrogen and phosphorus stocks (Keller et al. 2007, Keuper et al. 2012). These nutrient stocks, and restricted flowpaths caused by permafrost, affect water chemistry of streams draining catchments underlain with permafrost. In areas of discontinuous permafrost, streams draining catchments underlain with extensive permafrost have higher concentration of recalcitrant DOM when compared to streams draining watersheds with little permafrost (Balcarczyk et al. 2009), due to various factors such as restricted contact with mineral soil limiting microbial DOM processing and nutrient turnover. Streams draining low permafrost watersheds have lower DOM concentration, but this DOM is more biologically available to organisms (Balcarczyk et al. 2009), presumably due to active microbial decomposition in unfrozen soils.

Permafrost extent can also affect catchment and riparian vegetation, which has direct implications for light availability to stream channels, and the lability of terrestrially derived

carbon inputs to streams. For example, landscapes underlain by permafrost are characterized by black spruce (*Picea mariana*), with shallow root systems limited by active layer depth. Spruce dominated forests release litter into streams with DOM characteristic of higher carbon to nutrient ratio (Mutschlecner et al. 2017) than DOM derived from deciduous forests. These deciduous forests are characterized by quaking aspen (*Populus tremuloides*) and Alaska paper birch (*Betula neoalaskana*). Recalcitrant, spruce-derived DOM is less susceptible to microbial decomposition than more labile, deciduous-derived DOM sources, affecting the microbial communities that rely on this carbon as an energy source.

As global climate changes and temperature in high latitudes continues to rise (ACIA 2004), permafrost thaw in the boreal forest will alter watershed flowpaths, and carbon and nutrient inputs into streams. Permafrost loss will increase groundwater recharge and groundwater contributions to stream flow (Walvoord et al. 2012), causing stream water chemistry to more closely reflect that of groundwater. Warmer soil due to loss of frozen ground will alter vegetation and soil conditions, allowing more decomposition and turnover of nutrient pools within the organic layer, with high likelihood of groundwater export into stream networks (Davidson and Janssens 2006). Higher erosion caused by reduced stream bank stability due to permafrost loss (Vonk et al. 2015) will increase nutrient export to streams through release of higher concentrations of organic matter, inorganic nutrients, and major ions into catchments (Kokelj and Jorgenson 2013).

Permafrost degradation has already resulted in changing concentrations and exports of organic carbon and nitrogen in boreal forest headwater streams (Jones et al. 2005), and following changes in other solute fluxes, phosphorus export in freshwater systems is also predicted to increase with permafrost degradation (Frey et al. 2007, Frey and McClelland 2009). With the

potential increase of phosphorus concentration in headwater streams of Arctic and subarctic watersheds, total dissolved phosphorus export in Siberian rivers is predicted to increase by ~30% by 2100 due to permafrost thaw (Frey et al. 2007), and this pattern will likely similarly affect many high-latitude streams. Even small changes in resources and nutrient availability to organisms at the base of the food web will have large implications for entire stream ecosystems, affecting nutrient and carbon transformations and export downstream.

1.3 Resource limitation in high-latitude streams

In high-latitude streams, nitrogen can often limit metabolic activity in stream biofilms. In the Fennoscandian Arctic, both autotrophic and heterotrophic biomass and productivity in forested headwater stream biofilms are persistently limited by inorganic nitrogen concentrations (Burrows et al. 2015, Burrows et al. 2017, Myrstener et al. 2018), as well as organic carbon lability. Similarly, biofilms colonized in Greenland streams were found to be nitrogen limited, while nutrient uptake experiments revealed whole-stream phosphorus limitation (Docherty et al. 2018). Biofilm productivity in tundra streams has also been found to be constrained by temperature and light availability (Myrstener et al. 2018). In Icelandic streams, temperature positively impacts autotrophic nutrient use efficiency (Hood et al. 2018).

In the Alaskan Arctic, however, phosphorus controls biofilm biomass accrual and function (Peterson et al. 1985). Phosphorus concentration also controls dissolved organic carbon uptake rate in the boreal forest of interior Alaska (Mutschlecner et al. 2017). In Alaskan high-latitude streams, slight increases in phosphorus concentration increased primary production, algal biomass, heterotrophic respiration, and nitrogen uptake (Peterson et al. 2002, Slavik et al. 2004). In the Kuparuk River, an increase in dissolved phosphorus concentration (10 μg PO_4^{3-} - P L^{-1})

resulted in algal growth increasing an order of magnitude (Peterson et al. 1985, Peterson et al. 2002, Slavik et al. 2004). This increased algal biomass led to an increase in primary production, which stimulated heterotrophic activity, suggesting that the main organic carbon energy source for heterotrophic organisms shifted from predominantly allochthonous carbon to include newly fixed autochthonous carbon (Peterson et al. 1985).

1.4 Biogeochemistry of the Caribou-Poker Creeks Research Watershed

In the region of discontinuous permafrost in Interior Alaska, headwater streams of the Caribou-Poker Creeks Research Watershed (CPCRW; Fig. 1.3; Table 1.1) are characterized by high nitrate concentration (Petrone et al. 2006) and low organic and inorganic phosphorus concentrations, whereas organic carbon quality and quantity is related to catchment permafrost extent (Balcarczyk et al. 2009, Mutschlecner et al. 2018) (Table 1.2). Phosphorus controls heterotrophic dissolved organic carbon uptake in headwater streams of the CPCRW (Mutschlecner et al. 2017). We hypothesized that autotrophic nutrient uptake and primary production are also phosphorus limited. How autotrophs and heterotrophs compete for resources, including phosphorus, within these boreal forest headwater streams, remains unclear.

The permafrost distribution in interior Alaska creates an ideal location to study the impacts of permafrost loss due to warming on the biogeochemistry and resource limitation of the microbial communities inhabiting high-latitude streams. In this study, I explored how the controls of autotrophic primary production and heterotrophic respiration impact competition for inorganic nutrients between autotrophs and heterotrophs by measuring (1) patch-scale resource limitation of autotrophic and heterotrophic biomass and metabolism of biofilms grown on nutrient diffusing substrata (NDS), and (2) reach-scale nutrient uptake parameters. By enriching

streams with labile carbon and inorganic nutrients while also manipulating light, at both the patch and reach scales, I was able to gain insight into how alleviation of limiting energy and nutrient resources may affect resource utilization by autotrophic and heterotrophic organisms, as well as the competition between them for resources. I also measured carbon bioavailability and general stream water chemistry parameters to determine what resources may be limiting heterotrophic and autotrophic growth and productivity at ambient conditions. I then predicted how changes in these resources might impact microbes as climate change progresses and permafrost continues to thaw in the boreal forest. Unraveling the complex dynamics that control autotrophic and heterotrophic resource utilization in these subarctic systems is vital to understanding how they will respond to permafrost degradation.

1.5 Literature Cited

ACIA. 2004. Impacts of a Warming Arctic: Arctic Climate Impact Assessment. Cambridge University Press:140.

Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry 94:255-270.

Battin, T. J., K. Besemer, M. M. Bengtsson, A. M. Romani, and A. I. Packmann. 2016. The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology 14:251-263.

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, and A. I. Packman. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geoscience 1:95-100.

Battin, T. J., W. T. Sloan, S. Kjelleberg, H. Daims, I. M. Head, T. P. Curtis, and L. Eberl. 2007. Microbial landscapes: new paths to biofilm research. Nature Reviews Microbiology 5:76-81.

Bernhardt, E. S., and G. E. Likens. 2002. Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology 83:1689-1700.

Besemer, K., G. Singer, C. Quince, E. Bertuzzo, W. Sloan, and T. J. Battin. 2013. Headwaters are critical reservoirs of microbial diversity for fluvial networks. Proceedings of the Royal Society Britain 280:1-8.

Burrows, R. M., E. R. Hotchkiss, M. Jonsson, H. Laudon, B. G. McKie, and R. A. Sponseller. 2015. Nitrogen limitation of heterotrophic biofilms in boreal streams. Freshwater Biology 60:1237-1251.

Burrows, R. M., H. Laudon, B. G. McKie, and R. A. Sponseller. 2017. Seasonal resource limitation of heterotrophic biofilms in boreal streams. Limnology and Oceanography 62:164-176.

Cotner, J. B., and R. G. Wetzel. 1992. Uptake of dissolved inorganic and organic phosphorus compounds by phytoplankton and bacteriaplankton. Limnology and Oceanography 37:232-243.

Cross, W. F., J. P. Benstead, P. C. Frost, and S. A. Thomas. 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50:1895-1912.

Cross, W. F., J. M. Hood, J. P. Benstead, A. D. Huryn, and D. Nelson. 2015. Interactions between temperature and nutrients across levels of ecological organization. Global Change Biology 21:1025-1040.

Currie, D. J. 1990. Large-scale variability and interactions among phytoplankton, bacterioplankton, and phosphorus. American Society of Limnology and Oceanography 35:1437-1455.

Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165-173.

Docherty, C. L., T. Riis, D. M. Hannah, S. R. Leth, and A. M. Milner. 2018. Nutrient uptake controls and limitation dynamics in north-east Greenland streams. Polar Research 37:1-12.

Dodds, W. K. 2007. Trophic state, eutrophication and nutrient criteria in streams. Trends in Ecology and Evolution 22:669-676.

Dodds, W. K., and V. H. Smith. 2016. Nitrogen, phosphorus, and eutrophication in streams. Inland Waters 6:155-164.

Elser, J. J., M. E. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135-1142.

Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs 43:421-439.

Frey, K. E., and J. W. McClelland. 2009. Impacts of permafrost degradation on arctic river biogeochemistry. Hydrological Processes 23:169-182.

Frey, K. E., J. W. McClelland, R. M. Holmes, and L. C. Smith. 2007. Impacts of climate warming and permafrost thaw on the riverine transport of nitrogen and phosphorus to the Kara Sea. Journal of Geophysical Research: Biogeosciences 112:1-10.

Hill, W. R., S. E. Fanta, and B. J. Roberts. 2009. Quantifying phosphorus and light effects in stream algae. Limnology and Oceanography 54:368-380.

Hood, J. M., J. P. Benstead, W. F. Cross, A. D. Huryn, P. W. Johnson, G. M. Gislason, J. R. Junker, D. Nelson, J. S. Olafsson, and C. Tran. 2018. Increased resource use efficiency amplifies positive response of aquatic primary production to experimental warming. Global Change Biology 24:1069-1084.

Jones, J. B., K. C. Petrone, J. C. Finlay, L. D. Hinzman, and W. R. Bolton. 2005. Nitrogen loss from watersheds of interior Alaska underlain with discontinuous permafrost. Geophysical Research Letters 32:1-4.

Kaplan, L. A., T. N. Wiegner, J. D. Newbold, P. H. Ostrom, and H. Gandhi. 2008. Untangling the complex issue of dissolved organic carbon uptake: a stable isotope approach. Freshwater Biology 53:855-864.

Keller, K., J. D. Blum, and G. W. Kling. 2007. Geochemistry of soils and streams on surfaces of varying ages in Arctic Alaska. Arctic, Antarctic, and Alpine Research 39:84-98.

Keuper, F., P. M. Bodegom, E. Dorrepaal, J. T. Weedon, J. Hal, R. S. P. Logtestijn, and R. Aerts. 2012. A frozen feast: thawing permafrost increases plant-available nitrogen in subarctic peatlands. Global Change Biology 18:1998-2007.

Kokelj, S. V., and M. T. Jorgenson. 2013. Advances in Thermokarst Research. Permafrost and Periglacial Processes 24:108-119.

Kuehn, K. A., S. N. Francoeur, R. H. Findlay, and R. K. Neely. 2014. Priming in the microbial landscape: periphytic algal stimulation of litter-associated microbial decomposers. Ecology 95:749-762.

Lock, M. A., R. R. Wallace, J. W. Costerton, R. M. Ventullo, and S. E. Charlton. 1984. River epilithon: toward a structural-funtional model. Nordic Society Oikos 42:10-22.

Lyon, D. R., and S. E. Ziegler. 2009. Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. Limnology and Oceanography 54:439-449.

Mann, P. J., W. V. Sobczak, M. M. Larue, E. Bulygina, A. Davydova, J. E. Vonk, J. Schade, S. Davydov, N. Zimov, R. M. Holmes, and R. G. Spencer. 2014. Evidence for key enzymatic controls on metabolism of Arctic river organic matter. Global Change Biology 20:1089-1100.

Marcarelli, A. M., H. A. Bechtold, A. T. Rugenski, and R. S. Inouye. 2009. Nutrient limitation of biofilm biomass and metabolism in the Upper Snake River basin, southeast Idaho, USA. Hydrobiologia 620:63-76.

Mutschlecner, A. E., J. J. Guerard, J. B. Jones, and T. K. Harms. 2017. Phosphorus Enhances Uptake of Dissolved Organic Matter in Boreal Streams. Ecosystems:675-688.

Mutschlecner, A. E., J. J. Guerard, J. B. Jones, and T. K. Harms. 2018. Regional and intra-annual stability of dissolved organic matter composition and biolability in high-latitude Alaskan rivers. Limnology and Oceanography 63:1605-1621.

Myrstener, M., G. Rocher-Ros, R. M. Burrows, A. K. Bergstrom, R. Giesler, and R. A. Sponseller. 2018. Persistent nitrogen limitation of stream biofilm communities along climate gradients in the Arctic. Global Change Biology:1-12.

Pastick, N. J., M. T. Jorgenson, B. K. Wylie, S. J. Nield, K. D. Johnson, and A. O. Finley. 2015. Distribution of near-surface permafrost in Alaska: Estimates of present and future conditions. Remote Sensing of Environment 168:301-315.

Pastor, A., A. Freixa, L. J. Skovsholt, N. Wu, A. M. Romani, and T. Riis. 2019. Microbial Organic Matter Utilization in High-Arctic Streams: Key Enzymatic Controls. Microbial Ecology:1-16.

Persson, J., P. Fink, A. Goto, J. M. Hood, J. Jonas, and S. Kato. 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos 119:741-751.

Peterson, B. J., J. E. Hobbie, A. E. Hershey, M. A. Lock, T. E. Ford, J. R. Vestal, V. L. McKinley, M. A. J. Hullar, M. C. Miller, R. M. Ventullo, and G. S. Volk. 1985.

Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science 229:1383-1386.

Peterson, B. J., R. M. Holmes, J. W. McClelland, C. J. Vorosmarty, R. B. Lammers, A. I. Shiklomanov, I. A. Shiklomanov, and S. Rahmstorf. 2002. Increasing river discharge to the Arctic Ocean. Science 298:2171-2173.

Petrone, K. C., J. B. Jones, L. D. Hinzman, and R. D. Boone. 2006. Seasonal export of carbon, nitrogen, and major solutes from Alaskan catchments with discontinuous permafrost. Journal of Geophysical Research: Biogeosciences 111:1-13.

Piper, L., W. F. Cross, and B. L. McGlynn. 2017. Colimitation and the coupling of N and P uptake kinetics in oligotrophic mountain streams Biogeochemistry 132:165-184.

Rasmussen, J. J., A. Baattrup-Pedersen, T. Riis, and N. Friberg. 2011. Stream ecosystem properties and processes along a temperature gradient. Aquatic Ecology 45:231-242.

Rier, S. T., J. M. Shirvinski, and K. C. Kinek. 2014. In situ light and phosphorus manipulations reveal potential role of biofilm algae in enhancing enzyme-mediated decomposition of organic matter in streams. Freshwater Biology 59:1039-1051.

Rier, S. T., and R. J. Stevenson. 2002. Effects of light, dissolved organic carbon, and inorganic nutrients on the relationship between algae and heterotrophic bacteria in stream periphyton. Hydrobiologia 489:179-184.

Risse-Buhl, U., N. Trefzger, A. G. Seifert, W. Schonborn, G. Gleixner, and K. Kusel. 2012. Tracking the autochthonous carbon transfer in stream biofilm food webs. FEMS Microbiology Ecology 79:118-131.

Robbins, C. J., R. S. King, A. D. Yeager, C. M. Walker, J. A. Back, R. D. Doyle, and D. F. Whigham. 2017. Low-level addition of dissolved organic carbon increases basal ecosystem function in a boreal headwater stream. Ecosphere 7:1-15.

Romani, A. M., and S. Sabater. 2001. Structure and activity of rock and sand biofilms in a mediterranean stream. Ecology 82:3232-3245.

Schade, J. D., K. MacNeill, S. A. Thomas, C. F. McNeely, J. R. Welter, J. Hood, M. Goodrich, M. E. Power, and J. C. Finlay. 2011. The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams. Freshwater Biology 56:424-436.

Slater, A. G., and D. M. Lawrence. 2013. Diagnosing present and future permafrost from climate models. Journal of Climate 26:5608-5623.

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey, and J. E. Hobbie. 2004. Long-term responses of the Kaparuk River ecosystem to phosphorus fertilization. Ecology 85:939-954.

Sterner, R. W., J. Clasen, W. Lampert, and T. Weisse. 1998. Carbon:phosphorus stoichiometry and food chain production. Ecology Letters 1:146-150.

Tank, J. L., and W. K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology 48:1031-1049.

Thorp, J. H., and M. D. Delong. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos 93:543-550.

Van Horn, D. J., R. L. Sinsabaugh, C. D. Takacs-Vesbach, K. R. Mitchell, and C. N. Dahm. 2011. Response of heterotrophic stream biofilm -communities to a gradient of resources. Aquatic Microbial Ecology 64:149-161.

Vonk, J. E., S. E. Tank, W. B. Bowden, I. Laurion, W. F. Vincent, P. Alekseychik, M. Amyot, M. F. Billet, J. Canário, R. M. Cory, B. N. Deshpande, M. Helbig, M. Jammet, J. Karlsson, J. Larouche, G. MacMillan, M. Rautio, K. M. Walter Anthony, and K. P. Wickland. 2015. Reviews and syntheses: Effects of permafrost thaw on Arctic aquatic ecosystems. Biogeosciences 12:7129-7167.

Walvoord, M. A., and R. G. Striegl. 2007. Increased groundwater to stream discharge from permafrost thawing in the Yukon River basin: Potential impacts on lateral export of carbon and nitrogen. Geophysical Research Letters 34:1-6.

Walvoord, M. A., C. I. Voss, and T. P. Wellman. 2012. Influence of permafrost distribution on groundwater flow in the context of climate-driven permafrost thaw: Example from Yukon Flats Basin, Alaska, United States. Water Resources Research 48:1-17.

Zhang, T., R. G. Barry, K. Knowles, J. A. Heginbottom, and J. Brown. 2008. Statistics and characteristics of permafrost and ground-ice distribution in the Northern Hemisphere. Polar Geography 31:47-68.

Ziegler, S. E., D. R. Lyon, and S. L. Townsend. 2009. Carbon release and cycling within epilithic biofilms in two contrasting headwater streams. Aquatic Microbial Ecology 55:285-300.

Zimov, S. A., E. A. Schuur, and F. S. Chapin. 2006. Permafrost and the global carbon budget. Science 312:1612-1613.

Figures

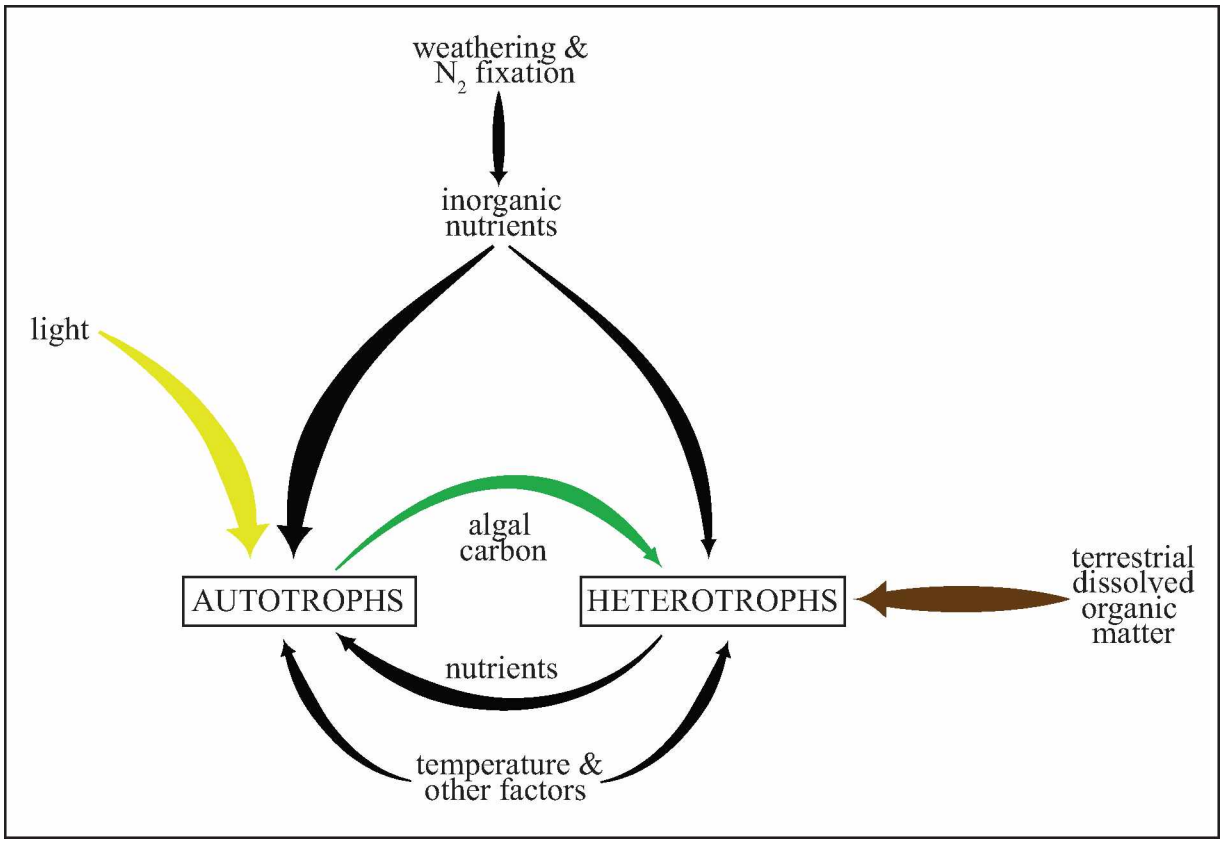

Figure 1.1. Conceptual diagram outlining controls on autotrophic primary production and heterotrophic respiration in boreal forest streams (modified from Currie 1990).

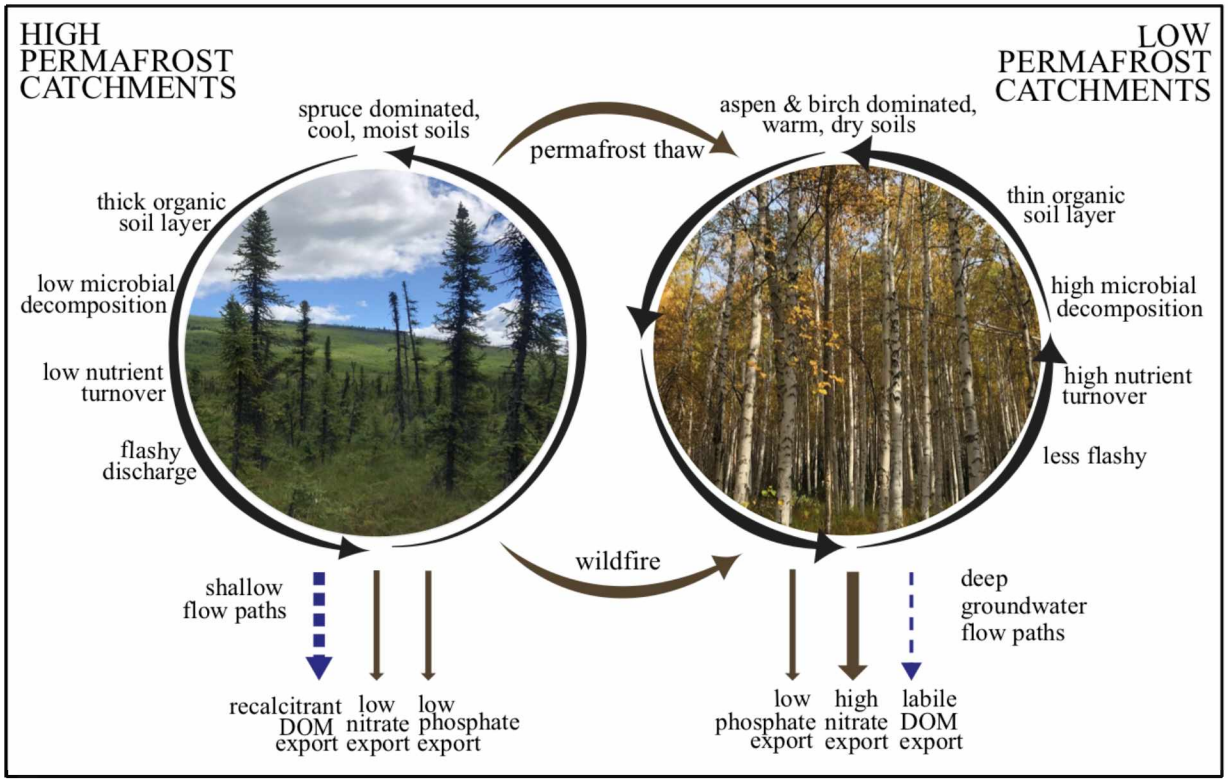

Figure 1.2. Impacts of permafrost on catchment and stream conditions within areas of discontinuous permafrost. High permafrost catchments are characterized by cool, moist soils with shallow flowpaths, and low microbial processing and nutrient turnover, while low permafrost catchments are characterized by warm, dry soils with deeper, groundwater flowpaths and high rates of microbial processing and nutrient turnover. As permafrost thaws, catchments will shift from spruce dominated landscapes to reflect characteristics of low permafrost watersheds with deciduous vegetation, thin organic soil layers, and high nitrate export.

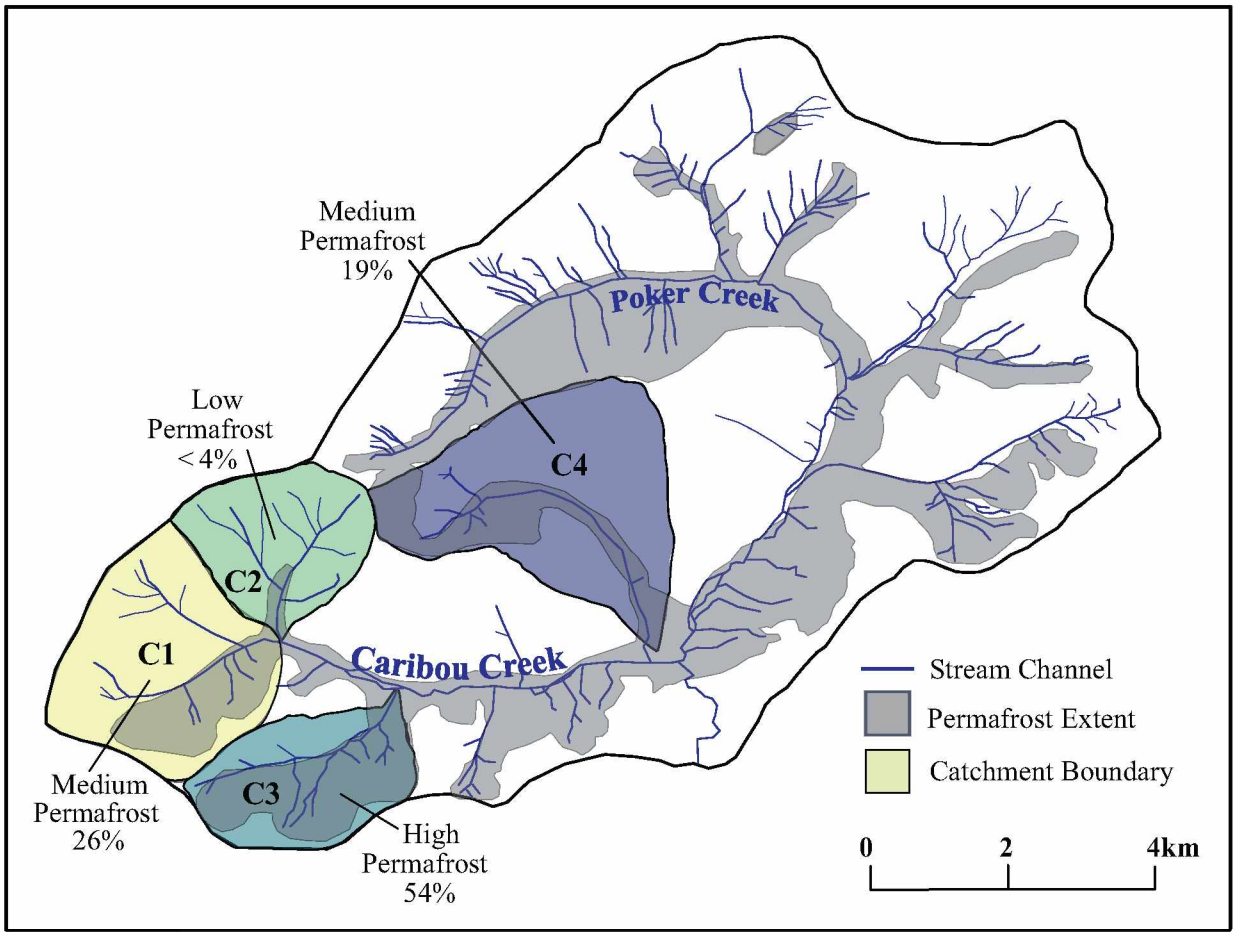

Figure 1.3. Caribou-Poker Creeks Research Watershed Map. Blue lines indicate stream channels, gray shaded regions represent land area underlain by permafrost, and colored regions represent stream catchments. Streams drain catchments that are underlain by < 4 to 53% permafrost extent.

Tables

Table 1.1. Catchment characteristics of the Caribou-Poker Creeks Research Watershed. Stream catchment area ranges from 5 to 10 km^2, elevation of all four streams is within 100 m, and catchment permafrost cover ranges from < 4 to 53%.

Stream	Catchment Area (km^2)	Elevation (m)	Aspect	Permafrost (% cover)
C1	6.7	325	E	26
C2	5.2	323	S	4
C3	5.7	274	NE	53
C4	10.0	226	SSE	19

Table 1.2. Water chemistry in headwater streams of the Caribou-Poker Creeks Research Watershed (CPCRW) during summer 2017 and 2018. Water chemistry was quantified from autosampler and mainstem water samples. The 2017 field season is included to show annual variation in physiochemical parameters at the CPCRW.

	Stream	Discharge ($L\ s^{-1}$)	Temp (^oC)	NO_3^- ($\mu g\ N\ L^{-1}$)	NH_4^+ ($\mu g\ N\ L^{-1}$)	PO_4^{3-} ($\mu g\ P\ L^{-1}$)	DOC ($mg\ C\ L^{-1}$)	$SUVA_{254}$ ($L\ mg\ C^{-1}\ m^{-1}$)
2018	C1	173.0*	3.62*	301.5	26.28	2.51	2.68	3.66
	C2	62.95	4.57	629.6	24.83	3.58	2.14	3.50
	C3	75.10	1.97	561.2	30.52	3.25	3.61	3.32
	C4	115.3	3.85	657.6	26.54	3.02	1.78	2.78
2017	C1	49.21*	2.25*	328.9	39.42	2.01	3.19	3.42
	C2	28.21	3.82	512.6	37.41	1.58	2.52	3.06
	C3	34.09	2.05	503.1	53.79	1.89	4.09	3.58
	C4	57.30	3.83	627.9	39.96	2.56	1.95	2.63

*C1 is the only stream where discharge and temperature are not measured continuously using pressure transducers throughout the summer field season; stream discharge was manually measured using slugs during August uptake experiments and temperature was measured biweekly during baseline sampling events.

Chapter 2: Resource limitation of autotrophs and heterotrophs in boreal forest headwater streams[1]

2.1 Abstract

Autotrophs and heterotrophs in stream biofilms dominate biogeochemical cycling and rely on nutrient and energy resources for growth and productivity. In the boreal forest, variation in these resources can originate from permafrost distribution and controls competition for nutrients between autotrophs and heterotrophs. We were interested in determining what resources control nutrient uptake by autotrophs and heterotrophs in headwater streams of interior Alaska, and how they affect competition for inorganic nutrients. We hypothesized that the competitive outcome would be dependent on availability of (i) organic carbon, (ii) light, or (iii) inorganic nutrients. To test our hypotheses, we measured resource limitation and competition at patch and reach scales with nutrient diffusing substrata and nutrient uptake experiments along a permafrost gradient in interior Alaska. At the patch scale, autotrophs were light and nutrient limited, whereas heterotrophs were carbon and nutrient limited. Heterotrophs also exhibited a larger response to nutrient enrichment, and outcompeted autotrophs for inorganic nutrients, when labile carbon concentration increased. At the reach scale, light had the largest influence on nutrient uptake. The positive response to increased nutrient and carbon availability suggests that the predicted increased export of nutrients and carbon into fluvial networks with permafrost degradation will impact biofilm structure and function, shifting to more heterotroph dominated patches as more labile carbon enters streams and heterotrophs outcompete autotrophs for inorganic nutrients. As permafrost thaws, and nutrients and organic

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carbon are mobilized into streams, nutrient uptake dynamics and competition between autotrophs and heterotrophs will be altered, affecting nutrient export.

2.2 Introduction

Stream biofilms dominate biogeochemical processes in stream channels (Battin et al. 2016) through nutrient transformations (Van Horn et al. 2011), extracellular enzyme production (Romani and Sabater 2001), and organic matter decomposition (Battin et al. 2008). Within biofilms, photoautotrophs (i.e., algae and cyanobacteria) require light to fix carbon (C) through photosynthesis (Hill 2009), while heterotrophic organisms (i.e., fungi, bacteria, and Archaea) use allocthonous or autochthonous-derived dissolved organic C (DOC) as an energy source for respiration (Bernhardt 2002, Robbins 2017). Both autotrophs and heterotrophs use inorganic nutrients for biosynthesis, while heterotrophs can additionally utilize organic nutrients. The availability of nutrient and organic C resources, along with abiotic controls such as light, control the competitive outcome between autotrophs and heterotrophs for inorganic nutrient within biofilms.

Because microbes must assimilate nutrients at relatively fixed ratios, nutrient stoichiometry of organic C, nitrogen (N), and phosphorus (P) in the water column impacts nutrient uptake rates (Schade et al. 2011, Piper et al. 2017), and nutrients can be co-limiting (Elser et al. 2007). Heterotrophs must maintain a fairly consistent nutrient stoichiometry, and organic C uptake is often limited by nutrient concentrations (Ziegler et al. 2009). A more plastic nutrient stoichiometry allows autotrophs to utilize limited inorganic nutrients (Sterner et al. 1998) if light is sufficient. In shaded channels with low primary production, heterotrophic bacteria and fungi predominantly use allochthonous organic matter for respiration (Kaplan et al.

2008); whereas in streams with open channels and higher light, autotrophic algae and cyanobacteria dominate organic C fixation and provide an autochthonous C source to heterotrophs for respiration (Thorp and Delong 2002, Dodds 2007). Temperature has both direct and indirect effects on metabolic rates, which in turn control nutrient use and assimilation (Rasmussen 2011 and Cross 2015). Therefore, in headwater streams, autotrophs and heterotrophs compete for inorganic nutrients with the competitive outcome dependent on light availability, labile dissolved organic matter (DOM) concentration, and temperature (Fig. 2.1).

In high-latitude streams surrounded by discontinuous permafrost, the extent of this perennially frozen ground impacts resources available to microbes. In interior Alaska and western Siberia, streams draining low permafrost or permafrost free catchments have higher phosphate and nitrate concentrations than streams draining catchments dominated by permafrost (Jones et al. 2005, Frey et al. 2007a, Frey et al. 2007b). Concentration and bioavailability of C reveals an opposite pattern, with higher DOC concentration observed in streams draining catchments underlain with higher permafrost extent, but a higher proportion of labile DOC in streams draining catchments with low permafrost extent (Balcarczyk et al. 2009). As climate warms and permafrost thaws, variation in resource availability (i.e. light, organic C) will likely be reflected as changes in nutrient fluxes and concentrations over time (Frey and McClelland 2009). For example, in the Kuparuk River in Arctic Alaska, a small increase in dissolved P concentration (10 μg PO_4^3 - P L^{-1}) increased algal biomass by an order of magnitude (Peterson et al. 1985, Peterson et al. 2002, Slavik et al. 2004). Coupled to increased algal biomass, GPP increased, resulting in increased heterotrophic respiration and microbial biomass, suggesting that heterotrophic microorganisms shifted from using allochthonous C to include newly fixed autochthonous C (Peterson et al. 1985).

In our research, we examined how variation in water chemistry and light availability affects the competition between autotrophs and heterotrophs in streams draining catchments with varying permafrost extent. The objectives of this study were to determine (1) which nutrient and energy resources control autotrophic and heterotrophic activity and biomass in boreal forest headwater steams, and (2) how these resources impact competition for inorganic nutrients between autotrophs and heterotrophs. We hypothesized that autotrophs and heterotrophs compete for inorganic nutrients with the outcome dependent on (i) DOM quality and quantity, (ii) light availability, or (iii) inorganic nutrient concentration (Fig. 2.1). To test our hypotheses, we used nutrient diffusing substrata (NDS) and nutrient uptake experiments with manipulations of light, inorganic nutrient concentrations, and availability of labile C. We also measured DOC bioavailability to determine C quality available to microbes in our study streams. Stream water chemistry was analyzed in parallel with NDS and uptake experimentation to determine how autotrophs and heterotrophs respond to varying ambient nutrient concentration.

2.3 Methods

2.3.1 Study site

Research was conducted at the Caribou-Poker Creeks Research Watershed (CPCRW; 65.15°N, 147.50°W) located 50 km NE of Fairbanks, AK, USA (Fig. 2.2). Part of the Bonanza Creek Long Term Ecological Research site (BNZ LTER), the CPCRW is a 104-km^2 research watershed underlain with discontinuous permafrost with no direct human influences other than scientific research. The climate is continental with cold winters (January mean -21°C), warm summers (July mean 16°C), and low annual precipitation (yearly average 411 mm). Stream temperature typically ranges from 2-5°C. Permafrost in the CPCRW is predominantly found on

north facing slopes due to solar aspect, and in poorly drained valley bottoms. Much of this frozen ground is unstable with permafrost temperature near the point of thawing. An estimated 2% of CPCRW permafrost has thawed over the past century (Hinzman et al. 2005).

Vegetation within the CPCRW is typical of interior Alaska. South facing slopes are dominated by hardwood forests of Alaska paper birch (*Betula neoalaskana*) and quaking aspen (*Populus tremuloides*) and have well-drained soils with shallow to deep inceptisols, thin organic horizons, and loamy texture. North facing slopes and valley bottoms are dominated by black spruce (*Picea mariana*) with moss and lichen understories, and are typically poorly drained gelisols with thick organic horizons underlain with permafrost. Alder are characteristic understory shrubs in uplands (*Alnus viridis sp. fruticosa*) and along stream channels in valley bottoms (*Alnus incana sp. tenuifolia*).

The headwater streams sampled in this study were all first-order (C1, C2, C3, and C4; Fig. 2.2) located in catchments varying in permafrost extent (4-53% cover; Table 2.1). Ambient water chemistry in the CPCRW varies with permafrost extent (Table 2.2). Streams draining catchments with high permafrost extent are characterized by higher nitrate and DOC concentration than streams draining catchments with lower permafrost extent (Petrone et al. 2006, Balcarczyk et al. 2009).

2.3.2 Study design

We examined resource limitation at the patch scale by deploying nutrient diffusing substrata (NDS). NDS were enriched with N, P, C, and combinations of these three elements, and deployed in shaded and non-shaded reaches of study streams. In non-shaded stream reaches, riparian vegetation was pruned around NDS incubation sites to permit maximum sunlight to

reach NDS within the channel. Shaded treatments were covered with tarps immediately upon deployment. After field deployment, NDS were collected and we conducted lab metabolism incubations and measured chlorophyll *a* (chl *a*) to quantify nutrient limitation and examine the competitive interactions between autotrophs and heterotrophs. We also measured P uptake at the reach scale using solute injections. Nutrient uptake was quantified during day and night time to separate autotrophic and heterotrophic processes. To determine ambient resource conditions within study streams, we quantified biodegradable labile DOC (LDOC) and nutrient concentrations. LDOC was quantified through 40-day lab incubations to measure C loss over time.

2.3.3 Nutrient diffusing substrata

NDS were constructed with 2% agar solution and amended with ammonium (0.5 M NH_4Cl, + N treatment), phosphate (0.5 M KH_2PO_4, + P treatment), acetate (0.5 M $C_2H_3NaO_2$, + C treatment), or combinations of these solutes (each at 0.5 M; + NP, + NPC). Control (U) treatments contained unamended agar. Acetate was chosen as a labile C source for heterotrophic organisms, and nutrient concentrations of 0.5 M were chosen to remain consistent with previous studies (Tank and Dodds 2003, Reisinger et al. 2016, Burrows et al. 2017, Myrstener et al. 2018). Nutrient amended agar for + NPC treatments were altered to contain 3% agar to accommodate higher concentrations of nutrient salts (Hauer and Lamberti 2011). All agar treatments were prepared in the laboratory, poured into 30 mL blue plastic cups (United States Plastics Corporation) with ~2.5 cm holes drilled into the center of the caps, and

allowed to cool and solidify. Once solidified, a fritted glass filter disk was placed directly on agar surface and cups were capped to keep disks in place.

Ten replicates of each NDS treatment in each stream (6 treatments, nonshaded and shaded; 120 NDS per stream; 60 shaded, 60 non-shaded) were securely attached to L-bars (grouped by shading, 60 NDS per L-bar cluster) with zip ties to prevent sample loss during in-stream incubations. Rebar was pounded into stream substrate and L-bars with attached NDS were secured to rebar with zip ties. NDS were positioned in clusters in the stream thalweg, approximately 15-30 cm under stream surface to ensure high light yet prevent exposure to air during low flow. NDS were deployed on July 6th and incubated for 20 days; NDS were checked every few days throughout incubation period to ensure NDS remained in place. Control treatments were situated upstream of nutrient treatments to avoid any passive diffusion of nutrients downstream of nutrient enriched NDS that might affect ambient water chemistry. Light meters (LI-COR 189, Lincoln, NE, USA) were used to ensure that tarps completely blocked photosynthetically active radiation (PAR) in shaded treatments (PAR < 0.6 μmol m^{-2} s^{-1}).

At the end of incubations, the fritted glass filter disks were removed from the agar surface of NDS cups and individually placed into 50 mL falcon tubes. Falcon tubes were then submerged in a bucket of fresh stream water to gently fill tubes. Tubes were placed in coolers for transport to the lab.

2.3.4 NDS metabolism incubations

Upon return to the lab, falcon tubes containing colonized biofilms and stream water were refrigerated (~4°C) overnight. The following day, fresh stream water was collected from Caribou Creek and transported to the lab for use during metabolism incubations. Metabolism

experiments were conducted in replicates of five for each treatment following protocol from Johnson et al. (2009b) and Reisinger et al. (2016). Stream water was saturated with oxygen by bubbling air before metabolism incubations to ensure high initial dissolved oxygen (DO) concentration. Falcon tubes containing colonized biofilms were then emptied and refilled with fresh DO-saturated stream water, capped under water to prevent air bubbles, and placed in coolers darkened with tarps (PAR < 0.5 μmol m^{-2} s^{-1}). DO was measured at the start and end of three-hour incubations using a handheld O_2 meter (YSI PROODO, Yellow Springs, OH, USA).

Within 12 hours of dark incubations, falcon tubes containing individual fritted glass filter disks were refilled with fresh unfiltered stream water, and the same protocol was followed under lit conditions. Samples were placed under full spectrum grow lights to simulate natural ambient light conditions (PAR = ~200-300 μmol m^{-2} s^{-1}) for additional three-hour incubations. All incubations were performed in the laboratory at room temperature (~19°C).

Respiration (μg O_2 cm^{-2} h^{-1}) was calculated as DO loss during dark incubations. Net ecosystem production (NEP; μg O_2 cm^{-2} h^{-1}) was calculated as the change in DO during light incubations. Gross primary production (GPP; μg O_2 cm^{-2} h^{-1}) was then calculated as:

$$GPP = NEP + R \quad (1)$$

where R is respiration. Falcon tubes containing stream water alone were included in lab incubations to quantify respiration and NEP occurring in the water column (n = 5); respiration and NEP measured from stream water incubated alone was subtracted from incubations of colonized biofilms. Immediately following metabolism incubations, samples were individually wrapped in aluminum foil and frozen at -80°C for chlorophyll *a* (chl *a*) analysis, which was completed within three months.

2.3.5 Nutrient uptake experiments

To determine how inorganic nutrient and labile C uptake varies in streams with differing water chemistry and light conditions, nutrient uptake experiments using tracer additions for spiraling curve characterization (TASCC) method of slug addition (Covino et al. 2010) were conducted in 2017 and 2018. Stream reaches of ~200 m were selected at each CPCRW headwater stream. Stream depths and widths were measured at ten transects along selected reaches. Discharge was measured before and after slug additions at the top and bottom of each reach. Travel time and discharge was measured one day prior to uptake experiments to prepare timing for nutrient releases by releasing a 300 g slug of sodium chloride (NaCl) and monitoring the peak in conductivity (YSI Pro30, Yellow Springs, OH, USA).

To measure uptake, we added a slug containing a conservative tracer (NaCl) and either a single nutrient (e.g., + P), or a combination of nutrients and acetate (+ NP or + NPC). We measured uptake of inorganic nutrients (+ P, + NP) in C1, C2, C3, and C4 streams in 2017, and uptake of inorganic nutrients and DOC (+ P, + NPC) in C1, C2, and C3 in 2018, under both light and dark conditions, resulting in eight nutrient uptake experiments in 2017 and nine nutrient uptake experiments in 2018. In 2018, nutrient uptake was measured in C1 and C2 on September 9, and in C3 on September 30. In 2017, all nutrient uptake experiments were conducted between August 10th and 25th. Because P concentration is low in CPCRW streams and is likely limiting (Mutschlecner et al. 2017), P alone was chosen as the only single nutrient used in uptake experiments. All uptake measurements within a stream were conducted within 24 hours to assure similar discharge throughout the experiment. Slugs containing nutrients were injected into streams as an instantaneous addition at the top of the chosen stream reach, and 25-30 water samples were collected in acid washed high-density polyethylene (HDPE) bottles from

the breakthrough curve (BTC) at the bottom of the reach. The targeted conservative tracer (NaCl) concentration was adjusted to be approximately 10 mg Cl^- L^{-1} above background concentration, whereas the mass of P was targeted to be 100 µg PO_4 - P L^{-1} above ambient conditions (Covino et al. 2010). N and acetate concentrations were then chosen based on the Redfield ratio and P concentration (Redfield 1958). Acetate concentration was altered (reduced) as needed to ensure complete solute dissolution in slug additions.

We measured heterotrophic nutrient uptake during the night and autotrophic and heterotrophic nutrient uptake in full sunlight. PAR was monitored throughout daytime and nighttime uptake experiments to ensure difference in light conditions (Odyssey PAR logger, Christchurch, NZ). During daytime, nutrient uptake under full light (PAR ~200-300 µmol m^{-2} s^{-1}) was sampled, and an additional nutrient uptake slug with the same nutrient additions was preformed after sunset (PAR < 1 µmol m^{-2} s^{-1}). Due to increased summer daylight in interior Alaska, nutrient uptake experiments were conducted in August and September to achieve complete darkness for a sufficient time to complete multiple uptake experiments. High precipitation during August and September limited nutrient uptake experiments in 2018 to CPCRW streams C1, C2 and C3. These streams were chosen due to location, differences in water chemistry, and discharge conditions.

2.3.6 Nutrient uptake calculations

Nutrient uptake length ($S_{w\text{-}amb}$), areal uptake rate (U_{amb}; Eq. 2), and uptake velocity ($V_{f\text{-}amb}$; Eq. 3) were calculated using the TASCC method (Covino et al. 2010). Ambient uptake length, $S_{w\text{-}amb}$, was estimated by plotting the linear regression of the added nutrient ($S_{w\text{-}add\text{-}int}$) against the nutrient concentration of each grab sample, and then calculating

the y-intercept. After determining $S_{w\text{-}amb}$ we were able to calculate ambient areal nutrient uptake (U_{amb}; Eq. 2) and ambient uptake velocity ($V_{f\text{-}amb}$; Eq. 3):

$$U_{amb} = Q\,[N_{amb}] / S_{w\text{-}amb}\,w \tag{2}$$

$$V_{f\text{-}amb} = U_{amb} / [N_{amb}] \tag{3}$$

where U_{amb} is the areal uptake at ambient conditions (μg m^{-2} min^{-1}), Q is stream discharge (m^3 s^{-1}), $[N_{amb}]$ is ambient nutrient concentration (μg L^{-3}), $S_{w\text{-}amb}$ is ambient uptake length (m), and w is wetted stream width (m). $V_{f\text{-}amb}$, or nutrient uptake velocity at ambient conditions for P and N (mm^{-2} min^{-1}), was calculated by dividing U_{amb} by $[N_{amb}]$, the concentration of the nutrient at ambient (μg L^{-3}).

2.3.7 LDOC incubations

We quantified DOC loss due to microbial decomposition to determine how DOC lability varied among streams. LDOC was measured at the start and end of NDS deployment. Four replicate water samples were collected in acid washed HDPE bottles from each stream's thalweg. All water samples were filtered through 1.0 μm glass fiber filters (Pall Corporation, Type A/E) upon return to the lab. Within 24 hours, samples were filtered to 0.22 μm (Whatman Nuclepore) to remove the microbial community. Used 0.22 μm filters were placed in nanopure water (ca. 10 mL nanopure for each filter), swirled, and allowed to soak to create a common microbial inoculum. Filtered water samples in volumes of 100 mL were then placed in 250 mL ashed glass incubation vials. All incubation vials were amended with nutrients to alleviate nutrient limitation of microbial decomposition in stream water by increasing ambient concentrations by

10 μM PO_4^{3-} and 80 μM NH_4^+ and NO_3^- (McDowell et al. 2006, Abbott et al. 2014). In order to ensure that all water samples were exposed to the same microbial community, 1 mL of the microbial inoculum was added to each incubation vial. Vials were then capped to eliminate water loss through evaporation and stored in the dark at room temperature. Once per week, caps were removed and vials were wafted to allow replenishment of oxygen into the head space of incubation vials to ensure no oxygen limitation of microbial processes.

To quantify C loss over time, we sampled 20 mL from each incubation vial at day zero, day eight, and day 40. Samples were collected from incubation vials and filtered to 0.22 μm to remove inoculated bacterial communities. Samples were then acidified with 2N HCl to remove inorganic C and to preserve samples until total organic C (TOC) quantification. Samples were placed in ashed glass scintillation vials, stored at room temperature (~ 19°C) in the dark, and quantified within three months. C loss was then calculated as change between initial and final TOC over time, averaged over the four incubation replicates. Any TOC remaining at day 40 was considered recalcitrant.

2.3.8 Water chemistry analysis

Ambient water chemistry data at each CPCRW stream was collected throughout the 2017 and 2018 field season as part of the BZN LTER database baseline sampling data collection. Autosamplers (ISCO, Lincoln, Nebraska, USA) were used to collect daily water samples, while additional grab samples were collected biweekly in HDPE bottles throughout the field season. Upon field collection, water samples were placed in a cooler, transported to the lab, and filtered to 1.0 μm within 24 hours. Water samples that could not be analyzed within five days were frozen for later analysis.

Water samples were analyzed for total dissolved P (TDP), soluble reactive P (SRP), total dissolved N (TDN), DOC, anions (Cl^-, NO_3^-, NO_2^-), cations (NH_4^+), specific ultraviolet absorbance ($SUVA_{254}$), and pH. We quantified SRP using the colorimetric molybdate blue method with a spectophotometer (Shimadzu, UVMini; 5 cm cell path, LOQ 0.7 μg P L^{-1}), and measured TDP as SRP following persulfate digestion (Murphy and Riley 1962). Total organic P (TOP) was calculated by taking the difference between TDP and SRP. TDN was measured following combustion to NO_x using a chemiluminescent N detector (Antek 720C) and Shimadza TOC 5000 analyzer (Merriam et al. 1996), which also quantified concentrations of DOC. Dissolved organic N (DON) was calculated as the difference between TDN and dissolved inorganic N (DIN; $NO_3^- + NO_2^- + NH_4^+$). We quantified anions and cations using ion chromatography (Thermo Scientific Dionex DX-320). SUVA was quantified by measuring absorbance at 254 nm (1 cm cell path; Shumadzu UVmini-1240). $SUVA_{254}$ was calculated by dividing UV absorbance at λ 254 nm by the DOC concentration (mg C L^{-1}) and is a measure of DOM aromaticity (Weishaar et al. 2003). Chl *a* was quantified using a spectrophotometer (Shimadzu, UVMini) after 24-h acetone extractions on ice (~2°C) (Hauer and Lamberti 2011).

For nutrient uptake, ammonium (NH_4^+) was quantified using the phenol hypochlorite method (Solorzano 1968) using automated colorimetry (SmartChem 170, Westco Scientific Instruments, LOQ 0.01 mg N L^{-1}). We quantified chloride (Cl^-) using the mercuric thiocyantate method (Zall et al. 1956). Samples with high SRP concentration (6.0-100 μg P L^{-1}) were measured using the colorimetric molybdate blue method (Synergy HT plate reader, Biotek; LOQ 5.15 μg P L^{-1}), while samples with lower SRP concentration (start and end of BTC; < 6.0 μg P L^{-1}) were measured using the same method on a spectrophotometer (Shimadzu, UVMini; 5 cm cell path, LOQ 0.7 μg P L^{-1}).

Stream discharge was calculated throughout the summer field season by continuously measuring stream stage height with pressure transducers (Solinst Levelogger, model 3001). Rating curves were created for each stream from biweekly slug velocity measurements and discharge was calculated from stage height data. Temperature was also continuously monitored in each stream throughout the field season (HOBO Conductivity logger). Continuous discharge was not collected at C1.

2.3.9 Statistical analysis

For NDS, we used two-way analysis of variance (ANOVA) to test for the nutrient limitation of each response variable (GPP, chl *a*, and respiration) in each stream, with nutrient treatment (+ N, + P, + C, + NP, + NPC, or U) and shading effect (shaded vs. ambient light) as the two factors (Tank and Dodds 2003, Reisinger et al. 2016). We used three-way ANOVA to determine whether responses varied between streams, with nutrient treatment, shading effect, and stream as the three factors. Tukey's honest significant difference (HSD) post-hoc comparisons were used to determine differences between treatments and shading effects in each stream ($\alpha < 0.05$).

To determine limitation of GPP, chl *a*, and respiration, we followed protocols developed by Tank and Dodds (2003). To determine nutrient limitation by a single nutrient (+ N, + P, or + C), treatments were compared to unamended controls (U). Primary limitation was determined if only one nutrient (e.g., + P) caused a significantly positive response. Streams were considered co-limited when multiple single nutrients (e.g., + P and + C) caused a positive response, but were not significantly different from each other, or when no single nutrient elicited a positive response but combinations of nutrient treatments (+ NP or + NPC) were significantly greater than

unamended controls. To determine secondary limitation, nutrient amendments with multiple nutrients (+ NP and + NPC) were compared to the primary limiting nutrient. Light was considered significantly limiting when the unamended control had a significantly higher response in light treatments than shaded treatments. Light was considered the primary control of autotrophic productivity or biomass when none of the shaded treatments showed a positive response to nutrient amendment, when compared to shaded unamended controls. Light was not considered to be primarily limiting when shaded treatments showed any positive response to nutrient amendment.

We calculated response ratios (RR) in order to normalize nutrient amended treatments to their paired control by dividing each response variable (GPP, chl *a*, respiration) for each treatment (+ N, + P, + C, + NP, + NPC) by the mean unamended control (U) of that variable in each stream (Burrows et al. 2017, Myrstener et al. 2018). Calculating the proportional change in biofilm GPP, chl *a*, and respiration allowed us to compare specific nutrient treatments and limitations across streams. Using RR, we ran a three-way ANOVA with stream, nutrient treatment, and shading effect as the three factors. If significant relationships ($\alpha < 0.05$) were detected, Tukey's HSD post hoc comparisons were used to determine significance levels between treatments and streams. RR were then compared for specific treatments across streams (e.g. C1 RR_{NPC} non-shaded vs C2 RR_{NPC} non-shaded). Simple linear regression (SLR) was used to determine relationships between biofilm response ratios and ambient water chemistry, physical parameters, or permafrost extent to further explore what is driving biofilm response to added nutrients.

We used SLR to examine the relationship between autotrophic biomass (chl *a*) and productivity (GPP) and to further explore trends in LDOC, water chemistry, and physical

parameters throughout the CPCRW during NDS incubations, uptake experiments, and the summer field season. All statistical analyses were performed in the statistical computing software R (Version 3.4.3: R project for statistical computing, Vienna, Austria).

2.4 Results

2.4.1 Stream water chemistry in the CPCRW in 2017 and 2018

Stream water chemistry varied among streams in 2017 and 2018 field seasons, but was relatively stable in each stream over the 20-day NDS incubation (Table 2.2). During the 2017 and 2018 field seasons, DOC concentration was highest in C3, the stream draining the highest permafrost catchment, ranging from 3.3 to 4.1 mg C L^{-1}. DOC and $SUVA_{254}$ were both lowest at C4. In contrast, nitrate was highest in C4, often exceeding 650 µg N L^{-1}. Nitrate concentration in C3 was lower than in C2 and C4 streams. C1 had the lowest nitrate concentration in the watershed, ranging from 260 to 329 µg N L^{-1}. SRP concentration remained low in all study streams, rarely exceeding 4 µg P L^{-1}, with little fluctuation by stream. Discharge varied with precipitation over field seasons, but was highest in C4, lowest in C2, and was flashiest, or most variable, in C3 (Table 2.2).

During NDS deployment, general stream chemistry remained stable during the 2018 field season. SRP concentration remained below 5 µg P L^{-1} in all streams (Table 2.2). Nitrate concentration was highest in C4, ranging from 621 µg N L^{-1} to 685 µg N L^{-1}, and was two-fold higher than in C1, which had a mean of 260 µg N L^{-1}. Ammonium concentration exhibited less variation than nitrate during NDS deployment, and remained below 25 µg N L^{-1} in all four streams. Temperature remained below 5°C with C3 consistently the coldest stream at 1.6°C. DOC concentration was variable among streams, but was lowest in C4. $SUVA_{254}$ was

also consistently lower in C4 when compared to the other three streams. Discharge varied over NDS incubations with precipitation. Mean discharge was highest in C4 at 105 L s^{-1}, which was almost twice as high as discharge in C2 at 59 L s^{-1}. Discharge was not measured at C1 during the NDS incubation period, but was relatively high when measured with slug injections in conjunction with uptake experimentation.

LDOC varied across streams (Table 2.3). C1 and C2 had the highest percent DOC loss over 40-day incubations at 19.1% and 14.4%, respectively (Table 2.3). C3 and C4, the streams with highest and lowest concentration of DOC, respectively, had almost no C loss over time, and measured C loss remained under 8% over 40-day incubations (Table 2.3).

2.4.2 Patch-scale autotrophic response to resource additions

Autotrophic GPP was highly influenced by light availability, and exhibited a smaller response to nutrient availability, but was highly variable across streams. Mean GPP ranged from 1.2 µg O_2 cm^{-2} h^{-1} in unamended control shaded treatments to 106.7 µg O_2 cm^{-2} h^{-1} in treatments exposed to light and amended with both inorganic N and P. Light availability during in-stream incubations was the primary limiting factor to autotrophic GPP (Fig. 2.4; Table 2.4). In all streams, non-shaded treatments had higher GPP than shaded treatments, with shaded treatments at or near zero. NPC was the sole treatment in which shaded and non-shaded GPP were not significantly different in all streams (C3; $p = 1$). In C4, NPC non-shaded treatments had approximately half the GPP rate as unamended controls ($p < 0.001$), with C3 showing a similar pattern of reduced GPP (Fig. 2.4). C3 and C4 both had lower GPP in non-shaded treatments containing a C amendment, when compared to non-shaded unamended controls. C1, C3, and C4

streams exhibited lower GPP rates in NPC treatments than NP treatments, however this trend was only significant in C4 ($p < 0.001$).

Autotrophic biomass, like GPP, was related to light availability. In all streams, chl *a* was consistently lower in shaded treatments than non-shaded treatments (Fig. 2.3). Unlike GPP, chl *a* exhibited a larger response to nutrient additions. In C1, chl *a* was elevated in all treatments containing P, but highest in combinations of inorganic N and P ($p = 0.018$). In C4, chl *a* was significantly higher in treatments containing inorganic N and P ($p < 0.001$). In C2 and C3, chl *a* had the largest positive response in treatments of inorganic P alone ($p < 0.001$ and $p = 0.19$, respectively), and in C2, chl *a* did not increase when N or acetate were added in addition to P. Across all streams, treatments containing acetate had lower autotrophic biomass accumulation than unamended controls, but this difference was only significant in C2 ($p = 0.036$). Algal growth in treatments containing N alone was also suppressed, but this pattern was only significant in C4 ($p < 0.001$). Similar to GPP, chl *a* was lower in NPC treatments than NP treatments in C3 and C4 streams ($p = 0.006$ and $p < 0.001$, respectively).

Autotrophic biomass and function of colonized biofilms, chl *a* and GPP respectively, were positively correlated ($r^2 = 0.63$, $p < 0.001$; Fig. 2.10). These parameters, however, were highly variable across streams, and C4 had higher chl *a* and GPP than any other CPCRW stream (Fig. 2.3 and 2.4), as well as more variation within treatments. GPP increased two-fold in C4 in all NDS treatments compared to C1, C2, and C3. C4 also had significantly higher accumulation of algal biomass than the other three streams for unamended control non-shaded treatments ($p < 0.01$). Mean chl *a* in C4 NPC amended treatments was 2.83 μg cm^{-2}, whereas the mean chl *a* measured in C1 and C2 NPC treatments were 1.11 μg cm^{-2} and 1.30 μg cm^{-2}, respectively. C1 and C2 had comparable chl *a* for all nutrient amendments (Fig. 2.3).

Nutrient treatments responded to nutrients proportionally the same across all streams and no patterns emerged in chl *a* RR (Fig. 2.6). For algal productivity, GPP, the response ratio of NPC (i.e. RR_{NPC}) in shaded treatments was positively correlated to total organic P ($r^2 = 0.56$, $p < 0.001$) and was significantly higher in C4 ($RR_{NPC} = 17.9$) than any other stream (C1 $RR_{NPC} = 1.3$; C2 $RR_{NPC} = 3.0$; and C3 $RR_{NPC} = 6.0$; $p < 0.001$; Fig. 2.7). For GPP in shaded treatments, RR_C, and RR_{NP} were also significantly higher in C4 than any other stream ($p < 0.001$; Fig. 2.7). We did not find any significant relationships between permafrost extent and RR of any nutrient amendment for algal biomass or productivity.

2.4.3 Patch-scale heterotrophic response to resource additions

Respiration varied with nutrient treatment in all CPCRW streams, but was most responsive to labile C addition. In shaded treatments, heterotrophic respiration only responded to acetate addition (Fig. 2.5; Table 2.4); this positive respiration response to a labile C source was significant in all streams for C alone ($p < 0.001$) and NPC amended treatments ($p < 0.001$). In non-shaded treatments, respiration increased in treatments with inorganic P and acetate, but was highest in treatments containing inorganic N, P, and acetate (Fig. 2.5; Table 2.4). Respiration increased to more than twice as high as unamended controls when both inorganic N and P were added in addition to acetate ($p < 0.001$). Respiration did not increase when inorganic N was the sole nutrient amendment, but in both C1 and C3, the streams with the highest ambient DOC concentration, respiration was significantly higher than unamended controls in treatments containing acetate alone ($p < 0.001$ and $p = 0.014$, respectively) and inorganic P alone ($p = 0.007$ and $p < 0.001$, respectively). In C2, the stream with the highest SRP concentration during NDS deployment, heterotrophic respiration significantly increased in

treatments with acetate ($p < 0.001$), but did not increase with addition of single inorganic nutrients (i.e. + N or + P). In C4, respiration was highest in treatments containing both inorganic N and P ($p = 0.036$), or inorganic N, P, and acetate ($p < 0.001$). C4 was the only stream where non-shaded respiration had a non-significant response to labile C addition ($p = 0.063$).

Respiration was higher in non-shaded treatments than shaded treatments in all streams, often significantly (Fig. 2.5). Treatments with acetate (both alone and in combination of inorganic nutrients) were more similar between shaded and non-shaded treatments than treatments amended with just inorganic nutrients. Respiration and GPP were positively correlated ($r^2 = 0.20, p = 0.001$). GPP:R was > 1 in the majority of non-shaded treatments. The exception was with the addition of acetate, where GPP:R was < 1 (Fig. 2.10). In general, shaded treatments GPP:R was < 1, with the exception of shaded unamended control and shaded P (Fig. 2.10).

Respiration RR differed by stream, but shaded NPC treatments had the greatest response. RR_{NPC} was significantly higher in C4 ($RR_{NPC} = 18.5$) than any other streams (C1 $RR_{NPC} = 6.92$; C2 $RR_{NPC} = 10.28$; C3 $RR_{NPC} = 12.1$; $p < 0.001$; Fig. 2.8). RR_C and RR_{NPC} of non-shaded treatments were both negatively correlated with total organic P concentration (RR_{NPC} $r^2 = 0.23$, $p = 0.03$; RR_C $r^2 = 0.49$, $p < 0.001$, respectively). In shaded treatments, RR_{NPC} was positively correlated with total organic P (RR_{NPC} $r^2 = 0.69$, $p < 0.001$), and total dissolved N concentration (RR_{NPC} $r^2 = 0.64$, $p < 0.001$). RR_P in shaded treatments was not positively correlated with any ambient water chemistry parameters or with permafrost extent. Excluding RR_{NP}, all respiration RR were positively correlated with LDOC (Fig. 2.11). RR_{NP} was the only treatment with a nonsignificant regression slope ($r^2 = 0.13, p = 0.119$). There was no relationship between permafrost extent and RR of any nutrient amendment for respiration.

2.4.4 Reach scale nutrient uptake

Nutrient uptake varied among streams and was most affected by light availability (Table 2.5). When P was the sole nutrient added, P ambient uptake length ($S_{w\text{-}amb}$; m) varied with light amendment but was similar across headwater streams and years. P ambient uptake length varied from 344 m to 557 m under light conditions in 2018 (Table 2.5). $S_{w\text{-}amb}$ of P in dark treatments were approximately twice as long as those performed under ambient light conditions, which corresponds to less efficient nutrient uptake. In 2018, adding N and C did not reduce ambient P uptake length, regardless of shading effect. Under dark conditions, PO_4^{3-} uptake was not detectable in C2. Uptake of NH_4^+ and acetate was not detectable in the study streams during the addition.

Measurements of ambient uptake velocity, $V_{f\text{-}amb}$ (mm^{-2} min^{-1}), and areal uptake, U_{amb} (μg P m^{-2} min^{-1}) showed similar patterns with little variation in response to nutrient amendment (Table 2.5). Variation was related with light condition (Table 2.5). Ambient uptake velocity and areal P uptake in treatments containing P alone was far more variable and often at least twice as high during uptake experiments performed under ambient light conditions ranging from 9.95 to 27.8 mm^{-2} min^{-1} and 20.2 to 42.9 μg P m^{-2} min^{-1}, respectively (Table 2.5). Conversely, under shaded conditions for nutrient treatments containing P alone, C3 $V_{f\text{-}amb}$ was 5.1 mm^{-2} min^{-1} and U_{amb} ranged from 11.7 μg P m^{-2} min^{-1} (Table 2.5).

2.5 Discussion

2.5.1 Nutrient limitation in boreal streams

Autotrophic and heterotrophic colonization and productivity responded positively to inorganic nutrient addition (Table 2.4) with P alleviating nutrient constraints on autotrophic biomass accumulation and heterotrophic metabolism (Fig. 2.3 and 2.5; Table 2.4). Our study adds to increasingly broad research describing P limitation in high-latitude headwater streams (Peterson et al. 1985, Corning et al. 1989, Diemer et al. 2015), although N limitation can also occur (Burrows et al. 2015, Myrstener et al. 2018). Inorganic nutrient limitation is not the only control, however, and biofilm biomass and productivity were largely constrained by a combination of resources in the CPCRW. The consistent P limitation that we observed was often in combination with other resources; organic C as a strong control of respiration and inorganic nutrient use by heterotrophs (Robbins et al. 2017), and light availability as a constraint of productivity and biomass accrual by autotrophs (Bernhardt and Likens 2004).

2.5.2 Resource limitation of autotrophs

In addition to inorganic nutrients, variation in light as an energy resource for autotrophs can also be a control of GPP, respiration, C limitation, and nutrient uptake in stream ecosystems (Huryn et al. 2014). Light was the primary control of autotrophic productivity and biomass accrual on NDS in all CPCRW headwater streams (Table 2.4), however, the positive response to light was not uniform across streams and nutrient treatments. For example, unamended NDS had approximately 40-65% higher autotrophic biomass and productivity in C4 than the other streams (Fig. 2.3 and 2.4). RR suggest that the mechanism driving this pattern in biomass affected each

nutrient treatment proportionally (Fig. 2.6). When treatment chl *a* was corrected to controls, ratios were close to one for all streams, meaning that treatments chl *a* did not differ from controls, and streams were not statistically different from each other. This lack of variation in RR between streams might be driven by accumulated light over the course of NDS in-stream deployment resulting from the southern aspect of the stream channel (Table 2.1). C4 flows south-southeast and therefore likely had the highest light exposure throughout NDS deployment. Temporal variation in light can impact autotrophic biomass accrual in response to nutrient enrichment (Myrstener et al. 2018), and light can have a larger effect on biomass accrual than nutrient concentration (Hill et al. 2009), supporting our prediction that higher chl *a* biomass is likely correlated with the increased light in C4.

In addition to light, nutrients also played a role in autotrophic biomass with increased chl *a* in all treatments containing inorganic P (Fig. 2.3). GPP, however, was only significantly limited by nutrients in one stream (Fig. 2.4C; Table 2.4). While inorganic nutrients may not have been significantly limiting to autotrophic productivity across the study streams, GPP was between 15-25% higher in treatments containing P than unamended treatments or treatments containing N alone (Fig. 2.4). While this limitation is not statistically significant, the trend suggests that nutrients, in addition to light, are indeed important drivers of autotrophic productivity in the CPCRW, a common finding among studies utilizing NDS in other streams (Tank and Dodds 2003, Johnson et al. 2009b, Reisinger et al. 2016, Myrstener et al. 2018).

2.5.3 Resource limitation of heterotrophs

Similar to autotrophs, heterotrophs can be highly responsive to resource gradients (Van Horn et al. 2011, Myrstener et al. 2018). Our results suggest that heterotrophic respiration in

CPCRW streams is regulated by both inorganic nutrients and labile C (Fig. 2.5; Table 2.4), and that these nutrient cycles are tightly coupled. In each stream, respiration responded positively to inorganic nutrient addition, yet responses increased by 100% or more when inorganic nutrients were introduced in combination with a labile C source (Fig. 2.5; Table 2.4). In the same CPCRW study steams, P uptake was directly coupled to C cycling (Mutschlecner et al. 2017). Nutrient uptake of DOC increased with the addition of inorganic P, suggesting that increasing P concentration in these headwater streams leads to higher DOC retention (Mutschlecner et al. 2017). Similar results were reported in Greenland streams, where DOC additions increased ammonium uptake (Docherty et al. 2018). While we found that labile C and inorganic nutrients are often co-limiting, our results suggest that labile C consistently limits heterotrophic respiration in the CPCRW (Fig. 2.5). This C limitation could be driven by a relatively recalcitrant C pool (Balcarczyk et al. 2009, Mutschlecner et al. 2018), which limits microbial decomposition of molecularly complex organic matter in these streams.

The resources controlling respiration also differed based on light availability during biofilm colonization (Fig. 2.5; Table 2.4). Respiration of biofilms colonized in light were consistently co-limited by inorganic nutrients and organic C, whereas respiration of biofilms in shaded reaches were controlled by organic C availability (Fig. 2.5), and only exhibited secondary inorganic nutrient limitation. This trend suggests that when heterotrophs are the sole colonizers within a biofilm (i.e. shaded treatments), they must solely rely on allochthonous C inputs, whereas when light is available, autochthonous C sources in the form of algal photosynthates are used by heterotrophs as a C source (Ziegler et al. 2009). We also found that respiration RR were positively correlated to LDOC (Fig. 2.11) in nonshaded treatments, regardless of nutrient amendment. This trend suggests that nutrient use efficiency increases with C lability, a finding

well supported in the literature (Ardón and Pringle 2007, Johnson et al. 2012). These results reiterate the importance of both inorganic nutrients and labile organic C in combination for heterotrophic growth, whether derived through autochthonous or allochthonous pathways (Rier and Stevenson 2002).

2.5.4 Competition driven by labile C

Autotrophs and heterotrophs rely on inorganic nutrients for GPP and respiration (Battin et al. 2016), and therefore compete within biofilms for these nutrients (Currie 1990). In the study streams, unamended treatment biofilms were autotrophic (GPP > R) under ambient stream conditions in light treatments, but heterotrophic (GPP < R) in shaded treatments (Fig. 2.10). When a labile C source was introduced, however, respiration increased to exceed GPP on unamended controls regardless of shading effect (Fig. 2.10), suggesting heterotrophic uptake of C and increased competition for inorganic nutrients.

Competition between autotrophs and heterotrophs for inorganic nutrients was most apparent in the suppression of autotrophic response to nutrient enrichment. For both GPP and chl *a*, nutrient amended treatments with organic C in addition to inorganic nutrients (+ NPC) exhibited reduced responses when compared to treatments with both inorganic N and P (Fig. 2.3C, D, and Fig. 2.4A, C, D). Because autotrophs cannot derive energy from organic C, chl *a* should not differ in treatments with both inorganic nutrients and C from treatments with only inorganic nutrients. The observed decrease in GPP suggests that heterotrophs may be outcompeting autotrophs for inorganic nutrients with the addition of labile C. Similarly, competition for nutrients between autotrophs and heterotrophs was further evidenced by lower algal biomass (chl *a*) with the addition of acetate, a finding that has been observed in other studies (Joint et al. 2002,

Stets and Cotner 2008, Bechtold et al. 2012). This finding supports our hypothesis ($\mathbf{H_1}$) that availability and lability of C controls competition between autotrophs and heterotrophs for inorganic nutrients. This pattern, however, was observed under high light and increased nutrient concentrations, suggesting that a combination of resources is controlling autotrophic and heterotrophic competition for inorganic nutrients. Algal suppression through competition for nutrients was most pronounced in C4, the stream with the highest autotrophic biomass accrual and productivity (Fig. 2.3D and Fig. 2.4D).

2.5.5 Reach scale resource limitation

Whereas autotrophic and heterotrophic biofilms at the patch scale were limited by various combinations of resources, uptake at the reach scale was largely affected by light. Based on uptake experiments where both nutrient combinations and light conditions were manipulated, the largest differences in P uptake were caused by presence and absence of light, suggesting that autotrophs are largely contributing to nutrient uptake at the reach scale. This finding is surprising, considering that most headwater streams are historically considered to be net heterotrophic (Vannote et al. 1980), with riparian vegetation suppressing autotrophic growth and rates of respiration exceeding autotrophic GPP. For our NDS experimentation, however, when riparian vegetation was removed and light was elevated, unamended control biofilms had a GPP:R >1, suggesting that biofilms exposed to full sunlight in the CPCRW may be autotroph-dominated under ambient conditions. Alternatively, this finding could be attributed to the use of inorganic substrata (fritted glass filter disks) for biofilm colonization, which may select for colonization of microbial biofilms dominated by autotrophs (Johnson et al. 2009b).

Increased labile C supply can alleviate nutrient limitation and therefore increase nutrient uptake by stream heterotrophs (Johnson et al. 2009a, Blaen et al. 2014, Docherty et al. 2018). We found evidence that acetate in conjunction with nutrient additions increased uptake of P under ambient light conditions. Previous findings suggest that P and C cycles are coupled in the CPCRW, further supporting our conclusions (Mutschlecner et al. (2017). These results, however, differed under dark conditions, when acetate addition did not enhance P uptake. Because we added C as labile acetate, it is surprising that C uptake was not measurable in conditions favoring heterotrophs. A lack of C uptake suggests that P limitation and N limitation may not have been alleviated.

Finding contrasting resource limitation at the patch and reach scale is not uncommon (Docherty et al. 2018, Tromboni et al. 2018), and suggests that only focusing on one scale may be misleading. For example, in tundra streams in Greenland, biofilms were NO_3^- limited at the patch scale, yet PO_4^{3-} limited at the reach scale (Docherty et al. 2018). Similarly, Tromboni et al. (2018) reached differing conclusions at each scale, suggesting that while the patch scale can allow us to categorize nutrient limitation, we cannot take whole-ecosystem variables into account until we measure nutrient limitation at the larger reach scale. By combining these methods to answer questions about resource limitation, we can gain insight about nutrient retention by autotrophs and heterotrophs, scaling up from small biofilm patches to entire stream reaches (Stets and Cotner 2008, Docherty et al. 2018, Griffiths and Johnson 2018, Tromboni et al. 2018).

2.5.6 Resource limitation of biofilms in a changing boreal forest

In boreal forest streams of Sweden and tundra streams of Greenland underlain by continuous permafrost, both autotrophic and heterotrophic biofilms have been found to be

persistently N limited at the patch scale (Burrows et al. 2015, Docherty et al. 2018, Myrstener et al. 2018). In addition, this N limitation often exists in conjunction with limitation of other resources, especially light and C (Burrows et al. 2017, Myrstener et al. 2018). Streams in Arctic and subarctic Alaska, however, are more frequently limited by P (Peterson et al. 1986, Slavik et al. 2004, Mutschlecner et al. 2017). In the Kuparuk river, for example, a slight increase in P concentration (10 mg L^{-1}) increased both algal and bacterial growth, and increased heterotrophic use of autochthonous C (Peterson et al. 1985). In streams of the CPCRW, P has been found to enhance stream retention of dissolved C (Mutschlecner et al. 2017), suggesting resource co-limitation.

As climate change reshapes regions with permafrost, and high-latitude watersheds warm, nutrient use in streams and biofilm communities will be altered. Increases in nutrient and C pools (Frey et al. 2007a, Reyes and Lougheed 2015), coupled with warming soils, will likely lead to increased productivity in streams. Our results indicate that boreal forest stream biofilms will become more productive, yet heterotrophs will likely outcompete autotrophs in the presence of labile C. This labile C source will mobilize into stream networks through permafrost degradation (Balcarczyk et al. 2009, Abbott et al. 2014), as the large C pools locked within this frozen ground become exposed (Zimov et al. 2006). Once mobilized, this labile C will likely be decomposed by heterotrophic microbes and released as CO_2 (Vonk et al. 2015), further contributing to permafrost-climate feedbacks (Schuur et al. 2015). Increasing stream temperature, which will ensue with permafrost degradation and increases in permafrost active layer depth, will likely positively affect autotrophic nutrient use efficiency (Cross et al. 2005) and primary production rates (Hood et al. 2018). These changes will also affect in-stream nutrient use and retention, altering biofilm functioning and nutrient export downstream.

Unraveling the complexity of resource utilization by and availability to stream autotrophs and heterotrophs will allow us to predict how headwater stream ecosystems will respond to changes in climate and ultimately permafrost loss in the boreal forest.

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2.7 Literature Cited

Abbott, B. W., J. R. Larouche, J. B. Jones, W. B. Bowden, and A. W. Balser. 2014. Elevated dissolved organic carbon biodegradability from thawing and collapsing permafrost. Journal of Geophysical Research: Biogeosciences 119:2049-2063.

Ardón, M., and C. M. Pringle. 2007. The quality of organic matter mediates the response of heterotrophic biofilms to phosphorus enrichment of the water column and substratum. Freshwater Biology 52:1762-1772.

Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry 94:255-270.

Battin, T. J., K. Besemer, M. M. Bengtsson, A. M. Romani, and A. I. Packmann. 2016. The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology 14:251-263.

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, and A. I. Packman. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geoscience 1:95-100.

Bechtold, H. A., A. M. Marcarelli, C. V. Baxter, and R. S. Inouye. 2012. Effects of N, P, and organic carbon on stream biofilm nutrient limitation and uptake in a semi-arid watershed. Limnology and Oceanography 57:1544-1554.

Bernhardt, E. S., and G. E. Likens. 2004. Controls on periphyton biomass in heterotrophic streams. Freshwater Biology 49:14-27.

Blaen, P. J., A. M. Milner, D. M. Hannah, J. E. Brittain, and L. E. Brown. 2014. Impact of Changing Hydrology on Nutrient Uptake in High Arctic Rivers. River Research and Applications 30:1073-1083.

Burrows, R. M., E. R. Hotchkiss, M. Jonsson, H. Laudon, B. G. McKie, and R. A. Sponseller. 2015. Nitrogen limitation of heterotrophic biofilms in boreal streams. Freshwater Biology 60:1237-1251.

Burrows, R. M., H. Laudon, B. G. McKie, and R. A. Sponseller. 2017. Seasonal resource limitation of heterotrophic biofilms in boreal streams. Limnology and Oceanography 62:164-176.

Corning, K. E., H. C. Duthie, and B. J. Paul. 1989. Phosphorus and glucose uptake by seston and epilithon in boreal forest streams. Journal of North American Benthological Society 8:123-133.

Covino, T. P., B. L. McGlynn, and R. A. McNamara. 2010. Tracer Additions for Spiraling Curve Characterization (TASCC): Quantifying stream nutrient uptake kinetics from ambient to saturation. Limnology and Oceanography: Methods 8:484-498.

Cross, W. F., J. P. Benstead, P. C. Frost, and S. A. Thomas. 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50:1895-1912.

Currie, D. J. 1990. Large-scale variability and interactions among phytoplankton, bacterioplankton, and phosphorus. American Society of Limnology and Oceanography 35:1437-1455.

Diemer, L. A., W. H. McDowell, A. S. Wymore, and A. S. Prokushkin. 2015. Nutrient uptake along a fire gradient in boreal streams of Central Siberia. Freshwater Science 34:1443-1456.

Docherty, C. L., T. Riis, D. M. Hannah, S. R. Leth, and A. M. Milner. 2018. Nutrient uptake controls and limitation dynamics in north-east Greenland streams. Polar Research 37:1-12.

Dodds, W. K. 2007. Trophic state, eutrophication and nutrient criteria in streams. Trends in Ecology and Evolution 22:669-676.

Elser, J. J., M. E. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135-1142.

Frey, K. E., and J. W. McClelland. 2009. Impacts of permafrost degradation on arctic river biogeochemistry. Hydrological Processes 23:169-182.

Frey, K. E., J. W. McClelland, R. M. Holmes, and L. C. Smith. 2007a. Impacts of climate warming and permafrost thaw on the riverine transport of nitrogen and phosphorus to the Kara Sea. Journal of Geophysical Research: Biogeosciences 112:1-10.

Frey, K. E., D. I. Siegel, and L. C. Smith. 2007b. Geochemistry of west Siberian streams and their potential response to permafrost degradation. Water Resources Research 43.

Griffiths, N. A., and J. T. Johnson. 2018. Influence of dual nitrogen and phosphorus additions on nutrient uptake and saturation kinetics in a forested headwater stream. Freshwater Science 37:810-825.

Hauer, F. R., and G. A. Lamberti. 2011. Methods in stream ecology. 2nd edition. Academic Press.

Hill, W. R., S. E. Fanta, and B. J. Roberts. 2009. Quantifying phosphorus and light effects in stream algae. Limnology and Oceanography 54:368-380.

Hinzman, L. D., N. D. Bettez, W. R. Bolton, F. S. Chapin, M. B. Dyurgerov, C. L. Fastie, B. Griffith, R. D. Hollister, A. Hope, H. P. Huntington, A. M. Jensen, G. J. Jia, T. Jorgenson, D. L. Kane, D. R. Klein, G. Kofinas, A. H. Lynch, A. H. Lloyd, A. D. McGuire, F. E. Nelson, W. C. Oechel, T. E. Osterkamp, C. H. Racine, V. E. Romanovsky, R. S. Stone, D. A. Stow, M. Sturm, C. E. Tweedie, G. L. Vourlitis, M. D. Walker, D. A. Walker, P. J. Webber, J. M. Welker, K. S. Winker, and K. Yoshikawa. 2005. Evidence and Implications of Recent Climate Change in Northern Alaska and Other Arctic Regions. Climatic Change 72:251-298.

Hood, J. M., J. P. Benstead, W. F. Cross, A. D. Huryn, P. W. Johnson, G. M. Gislason, J. R. Junker, D. Nelson, J. S. Olafsson, and C. Tran. 2018. Increased resource use efficiency amplifies positive response of aquatic primary production to experimental warming. Global Change Biology 24:1069-1084.

Huryn, A. D., J. P. Benstead, and S. M. Parker. 2014. Seasonal changes in light availability modify the temperature dependence of ecosystem metabolism in an arctic stream. Ecology 95:2826-2839.

Johnson, L. T., T. V. Royer, J. M. Edgerton, and L. G. Leff. 2012. Manipulation of the Dissolved Organic Carbon Pool in an Agricultural Stream: Responses in Microbial Community Structure, Denitrification, and Assimilatory Nitrogen Uptake. Ecosystems 15:1027-1038.

Johnson, L. T., J. L. Tank, and C. P. Arango. 2009a. The effect of land use on dissolved organic carbon and nitrogen uptake in streams. Freshwater Biology 54:2335-2350.

Johnson, L. T., J. L. Tank, and W. K. Dodds. 2009b. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. Canadian Journal of Fisheries and Aquatic Sciences 66:1081-1094.

Joint, I., P. Henriksen, G. A. Fonnes, D. Bourne, T. F. Thingstad, and B. Riemann. 2002. Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquatic Microbial Ecology 29:145-159.

Jones, J. B., K. C. Petrone, J. C. Finlay, L. D. Hinzman, and W. R. Bolton. 2005. Nitrogen loss from watersheds of interior Alaska underlain with discontinuous permafrost. Geophysical Research Letters 32:1-4.

Kaplan, L. A., T. N. Wiegner, J. D. Newbold, P. H. Ostrom, and H. Gandhi. 2008. Untangling the complex issue of dissolved organic carbon uptake: a stable isotope approach. Freshwater Biology 53:855-864.

McDowell, W. H., A. Zsolnay, J. A. Aitkenhead-Peterson, E. G. Gregorich, D. L. Jones, D. Jödemann, K. Kalbitz, B. Marschner, and D. Schwesig. 2006. A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biology and Biochemistry 38:1933-1942.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytical chemica acta 1:31-36.

Mutschlecner, A. E., J. J. Guerard, J. B. Jones, and T. K. Harms. 2017. Phosphorus Enhances Uptake of Dissolved Organic Matter in Boreal Streams. Ecosystems:675-688.

Mutschlecner, A. E., J. J. Guerard, J. B. Jones, and T. K. Harms. 2018. Regional and intra-annual stability of dissolved organic matter composition and biolability in high-latitude Alaskan rivers. Limnology and Oceanography 63:1605-1621.

Myrstener, M., G. Rocher-Ros, R. M. Burrows, A. K. Bergstrom, R. Giesler, and R. A. Sponseller. 2018. Persistent nitrogen limitation of stream biofilm communities along climate gradients in the Arctic. Global Change Biology:1-12.

Peterson, B. J., J. E. Hobbie, and T. L. Corliss. 1986. Carbon flow in a tundra stream ecosystem. Canadian Journal of Fisheries and Aquatic Sciences 43:1259-1270.

Peterson, B. J., J. E. Hobbie, A. E. Hershey, M. A. Lock, T. E. Ford, J. R. Vestal, V. L. McKinley, M. A. J. Hullar, M. C. Miller, R. M. Ventullo, and G. S. Volk. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science 229:1383-1386.

Peterson, B. J., R. M. Holmes, J. W. McClelland, C. J. Vorosmarty, R. B. Lammers, A. I. Shiklomanov, I. A. Shiklomanov, and S. Rahmstorf. 2002. Increasing river discharge to the Arctic Ocean. Science 298:2171-2173.

Petrone, K. C., J. B. Jones, L. D. Hinzman, and R. D. Boone. 2006. Seasonal export of carbon, nitrogen, and major solutes from Alaskan catchments with discontinuous permafrost. Journal of Geophysical Research: Biogeosciences 111:1-13.

Piper, L., W. F. Cross, and B. L. McGlynn. 2017. Colimitation and the coupling of N and P uptake kinetics in oligotrophic mountain streams Biogeochemistry 132:165-184.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. American Scientist 46:205-221.

Reisinger, A. J., J. L. Tank, and M. M. Dee. 2016. Regional and seasonal variation in nutrient limitation of river biofilms. Freshwater Science 35:474-489.

Reyes, F. R., and V. L. Lougheed. 2015. Rapid Nutrient Release from Permafrost Thaw in Arctic Aquatic Ecosystems. Arctic, Antarctic, and Alpine Research 47:35-48.

Rier, S. T., and R. J. Stevenson. 2002. Effects of light, dissolved organic carbon, and inorganic nutrients on the relationship between algae and heterotrophic bacteria in stream periphyton. Hydrobiologia 489:179-184.

Robbins, C. J., R. S. King, A. D. Yeager, C. M. Walker, J. A. Back, R. D. Doyle, and D. F. Whigham. 2017. Low-level addition of dissolved organic carbon increases basal ecosystem function in a boreal headwater stream. Ecosphere 7:1-15.

Romani, A. M., and S. Sabater. 2001. Structure and activity of rock and sand biofilms in a mediterranean stream. Ecology 82:3232-3245.

Schade, J. D., K. MacNeill, S. A. Thomas, C. F. McNeely, J. R. Welter, J. Hood, M. Goodrich, M. E. Power, and J. C. Finlay. 2011. The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams. Freshwater Biology 56:424-436.

Schuur, E. A., A. D. McGuire, C. Schadel, G. Grosse, J. W. Harden, D. J. Hayes, G. Hugelius, C. D. Koven, P. Kuhry, D. M. Lawrence, S. M. Natali, D. Olefeldt, V. E. Romanovsky, K. Schaefer, M. R. Turetsky, C. C. Treat, and J. E. Vonk. 2015. Climate change and the permafrost carbon feedback. Nature 520:171-179.

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey, and J. E. Hobbie. 2004. Long-term responses of the Kaparuk River ecosystem to phosphorus fertilization. Ecology 85:939-954.

Solorzano, L. 1968. Determination of ammonia in natural waters by the phenohypochlorite method. Limnology and Oceanography 14:799-801.

Sterner, R. W., J. Clasen, W. Lampert, and T. Weisse. 1998. Carbon:phosphorus stoichiometry and food chain production. Ecology Letters 1:146-150.

Stets, E. G., and J. B. Cotner. 2008. The influence of dissolved organic carbon on bacterial phosphorus uptake and bacterial phytopankton in two Minnesota lakes. Limnology and Oceanography 53:137-147.

Tank, J. L., and W. K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology 48:1031-1049.

Thorp, J. H., and M. D. Delong. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos 93:543-550.

Tromboni, F., S. A. Thomas, B. Gücker, V. Neres-Lima, C. Lourenço-Amorim, T. P. Moulton, E. F. Silva-Junior, R. Feijó-Lima, I. G. Boëchat, and E. Zandonà. 2018. Nutrient Limitation and the Stoichiometry of Nutrient Uptake in a Tropical Rain Forest Stream. Journal of Geophysical Research: Biogeosciences 123:2154-2167.

Van Horn, D. J., R. L. Sinsabaugh, C. D. Takacs-Vesbach, K. R. Mitchell, and C. N. Dahm. 2011. Response of heterotrophic stream biofilm -communities to a gradient of resources. Aquatic Microbial Ecology 64:149-161.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Vonk, J. E., S. E. Tank, P. J. Mann, R. G. M. Spencer, C. C. Treat, R. G. Striegl, B. W. Abbott, and K. P. Wickland. 2015. Biodegradability of dissolved organic carbon in permafrost soils and aquatic systems: a meta-analysis. Biogeosciences 12:6915-6930.

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science and Technology 37:4702-4708.

Zall, D. M., D. Fisher, and M. Garner. 1956. Photometric determination of chlorides in water. Analytical Chemistry 28:1655-1668.

Ziegler, S. E., D. R. Lyon, and S. L. Townsend. 2009. Carbon release and cycling within epilithic biofilms in two contrasting headwater streams. Aquatic Microbial Ecology 55:285-300.

Zimov, S. A., S. P. Davydov, G. M. Zimova, A. I. Davydova, E. A. G. Schuur, K. Dutta, and F. S. Chapin. 2006. Permafrost carbon: Stock and decomposability of a globally significant carbon pool. Geophysical Research Letters 33:1-5.

Figures

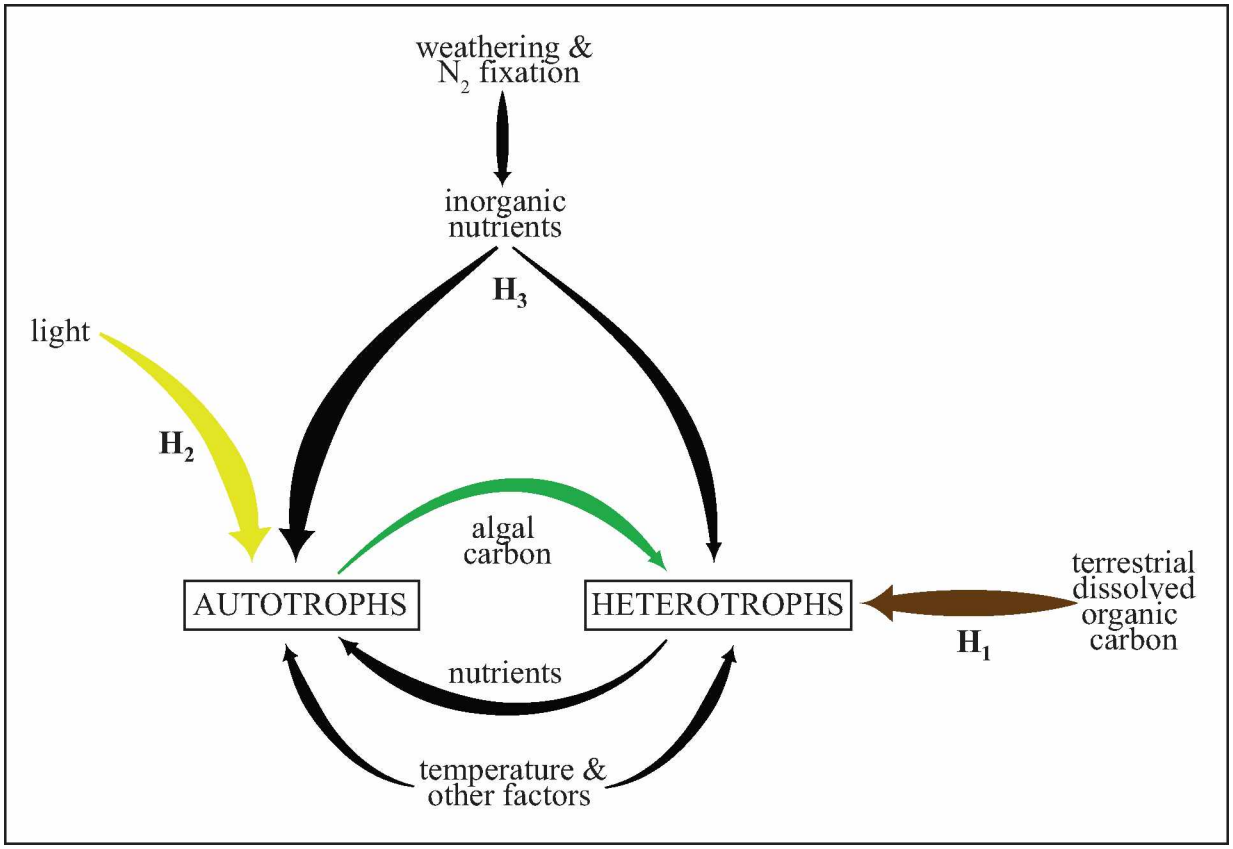

Figure 2.1. Controls on autotrophic primary production and heterotrophic respiration in boreal forest streams (modified from Currie 1990). Experimental hypotheses predict that autotrophic and heterotrophic microorganisms compete for inorganic nutrients (**H_3**) with the outcome of competition dependent on the quality and quantity of DOM (**H_1**) and light availability (**H_2**).

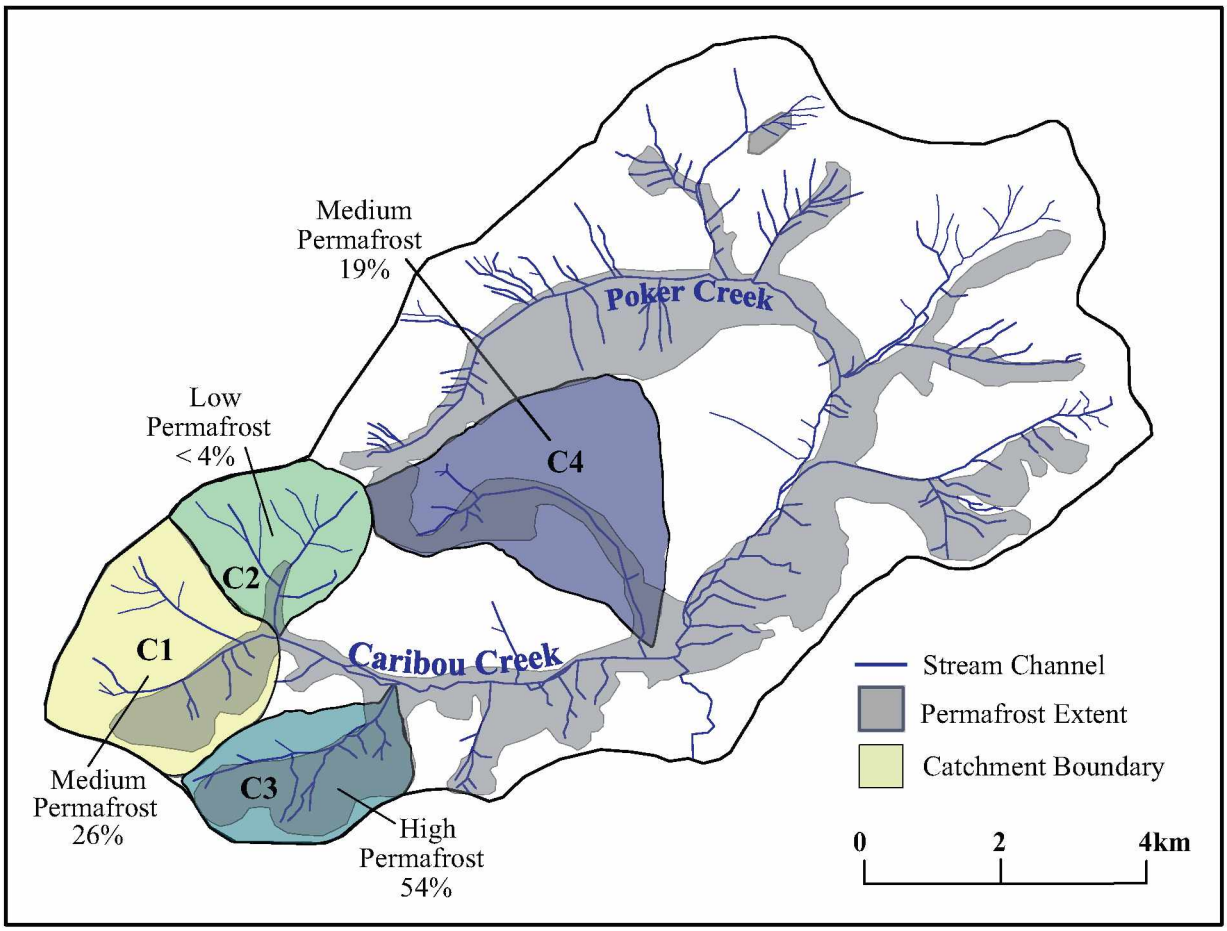

Figure 2.2. Caribou-Poker Creeks Research Watershed Map. Blue lines indicate stream channels, gray shaded regions represent land area underlain by permafrost, and colored regions represent study stream catchments.

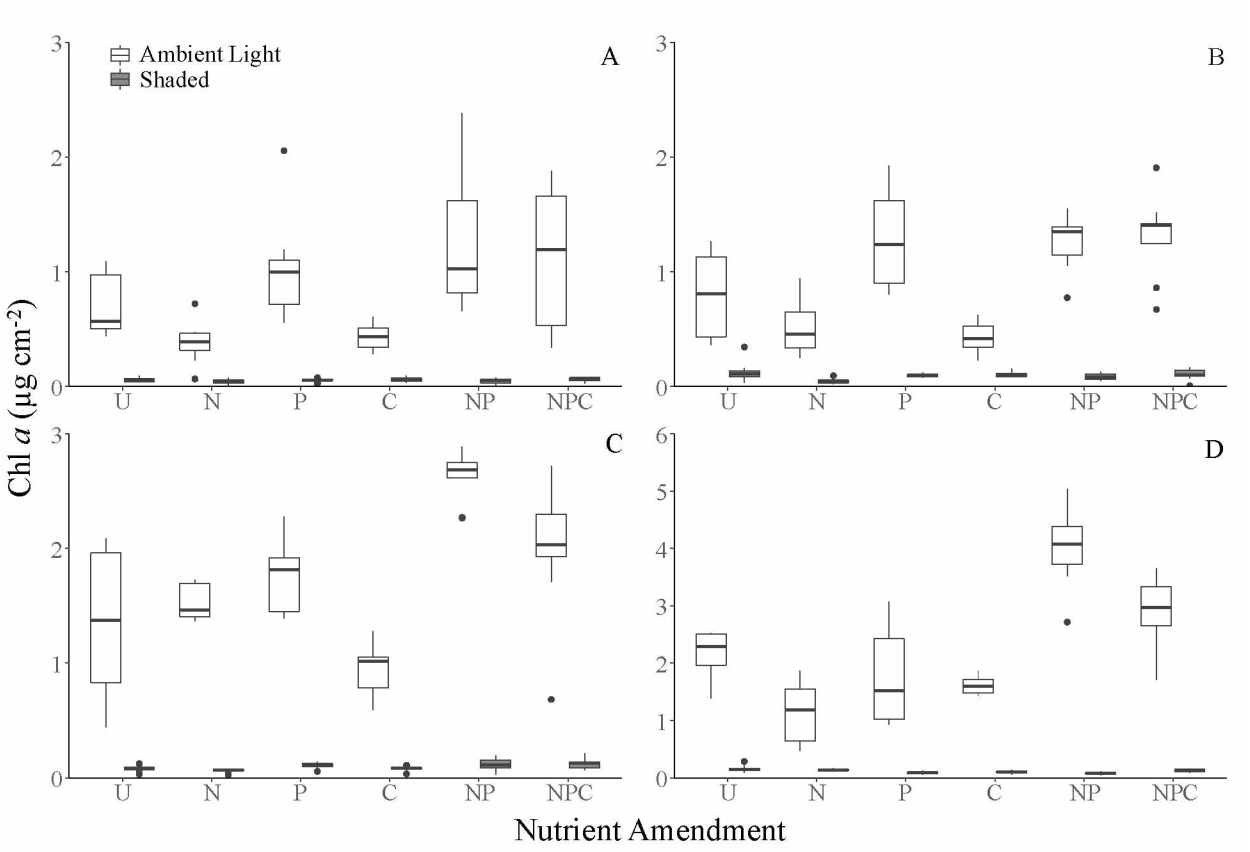

Figure 2.3. Chlorophyll *a* (chl *a*) quantified from biofilms colonized on nutrient diffusing substrata (NDS) at C1 (panel A), C2 (panel B), C3 (panel C), and C4 (panel D). Nutrient amendments include unamended control (U), ammonium (N), phosphorus (P), acetate (C), and combinations of these (NP, NPC). White boxes correspond to non-shaded treatments and dark boxes correspond to shaded treatments during in-stream NDS deployments. The center lines, box extent, error bars and points indicate the 50th percentile (median), the 25th and 75th percentile (interquartile range), the 95% confidence intervals, and outliers, respectively. Results of ANOVA with *p*-values from Tukey's HSD test are found in Table 2.4.

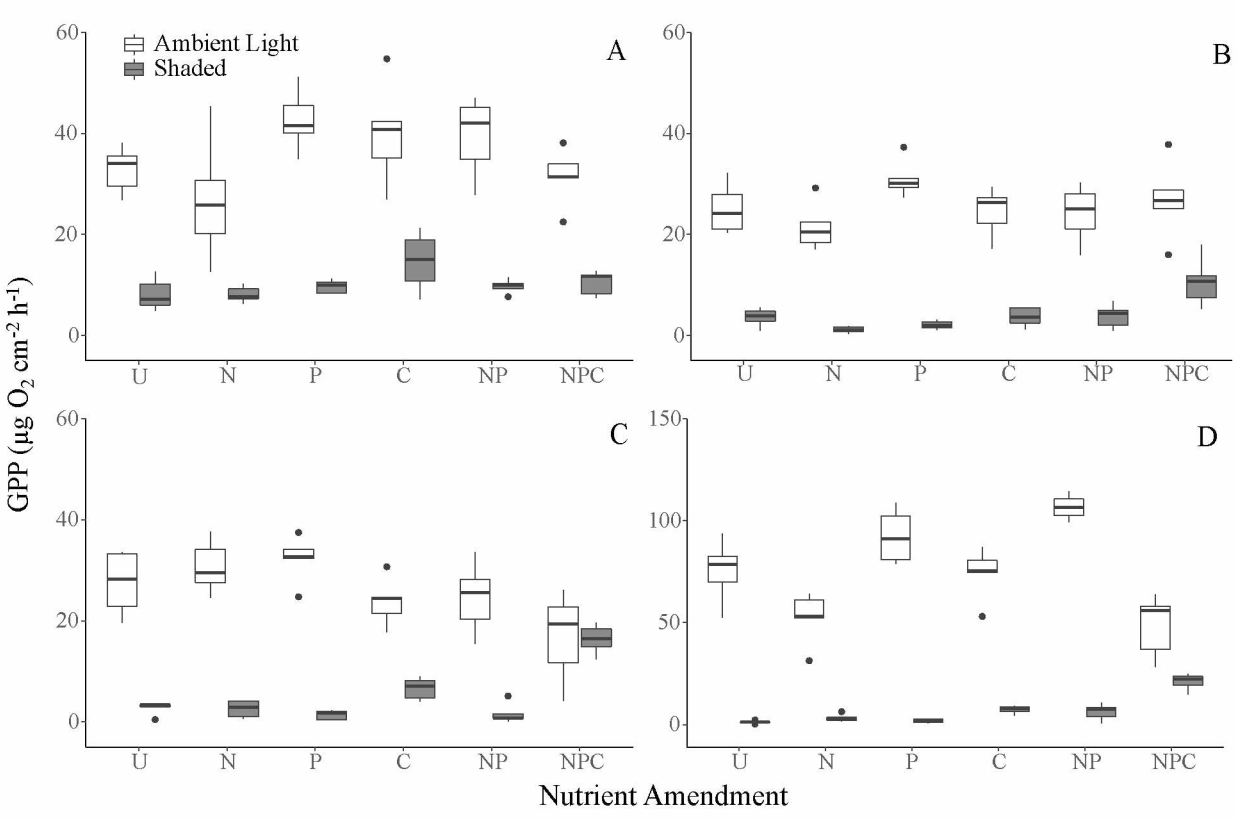

Figure 2.4. Gross primary production (GPP) of biofilms colonized during in-stream nutrient diffusing substrata (NDS) deployments in C1 (panel A), C2 (panel B), C3 (panel C), and C4 (panel D) streams. Nutrient amendments include unamended control (U), ammonium (N), phosphorus (P), acetate (C), and combinations of these (NP, NPC). White boxes correspond to non-shaded treatments and dark boxes correspond to shaded treatments during in-stream NDS deployments. The center lines, box extent, error bars and points indicate the 50th percentile (median), the 25th and 75th percentile (interquartile range), the 95% confidence intervals, and outliers, respectively. Results of ANOVA with *p*-values from Tukey's HSD test are found in Table 2.4.

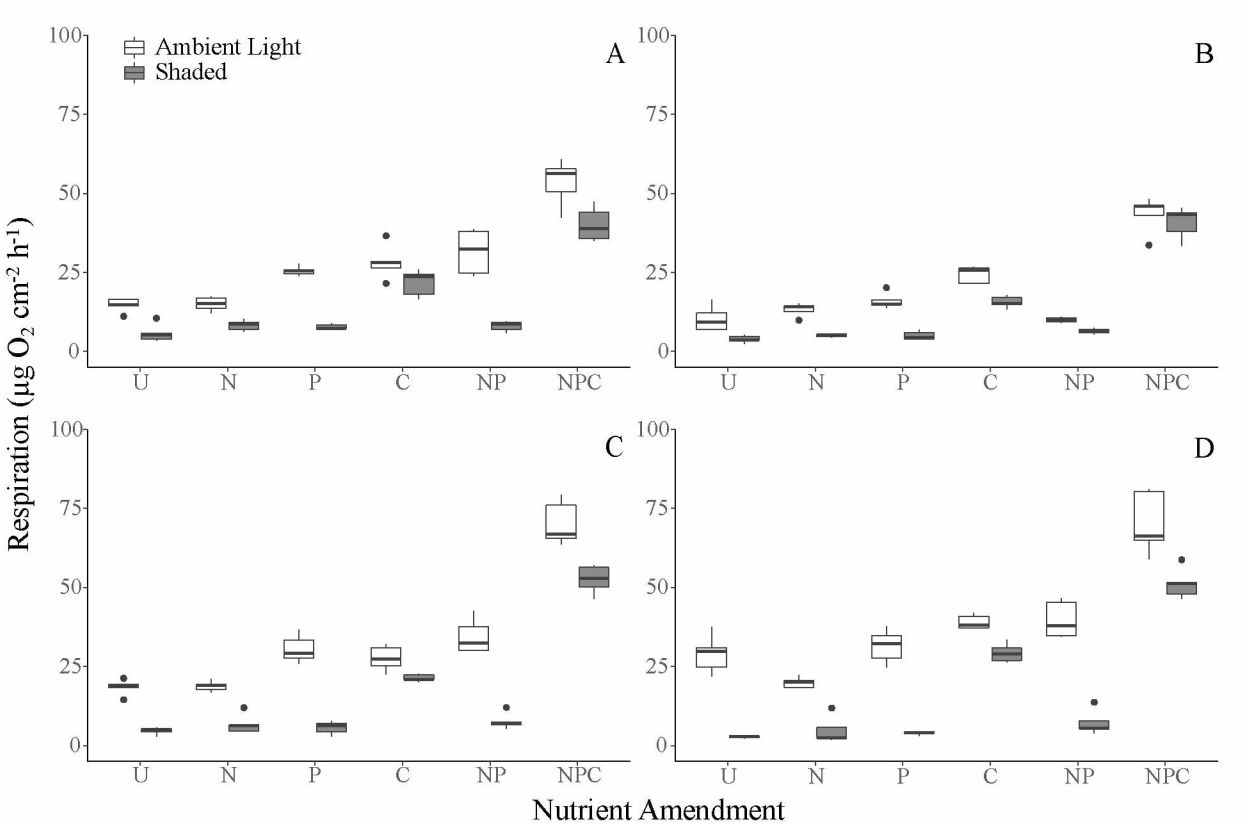

Figure 2.5. Respiration rates of biofilms colonized during in-stream nutrient diffusing substrata (NDS) deployments at C1 (panel A), C2 (panel B), C3 (panel C), and C4 (panel D). Nutrient amendments include unamended control (U), ammonium (N), phosphorus (P), acetate (C), and combinations of these (NP, NPC). White boxes correspond to non-shaded treatments and dark boxes correspond to shaded treatments during in-stream NDS deployments. The center lines, box extent, error bars and points indicate the 50th percentile (median), the 25th and 75th percentile (interquartile range), the 95% confidence intervals, and outliers, respectively. Results of ANOVA with *p*-values from Tukey's HSD test are found in Table 2.4.

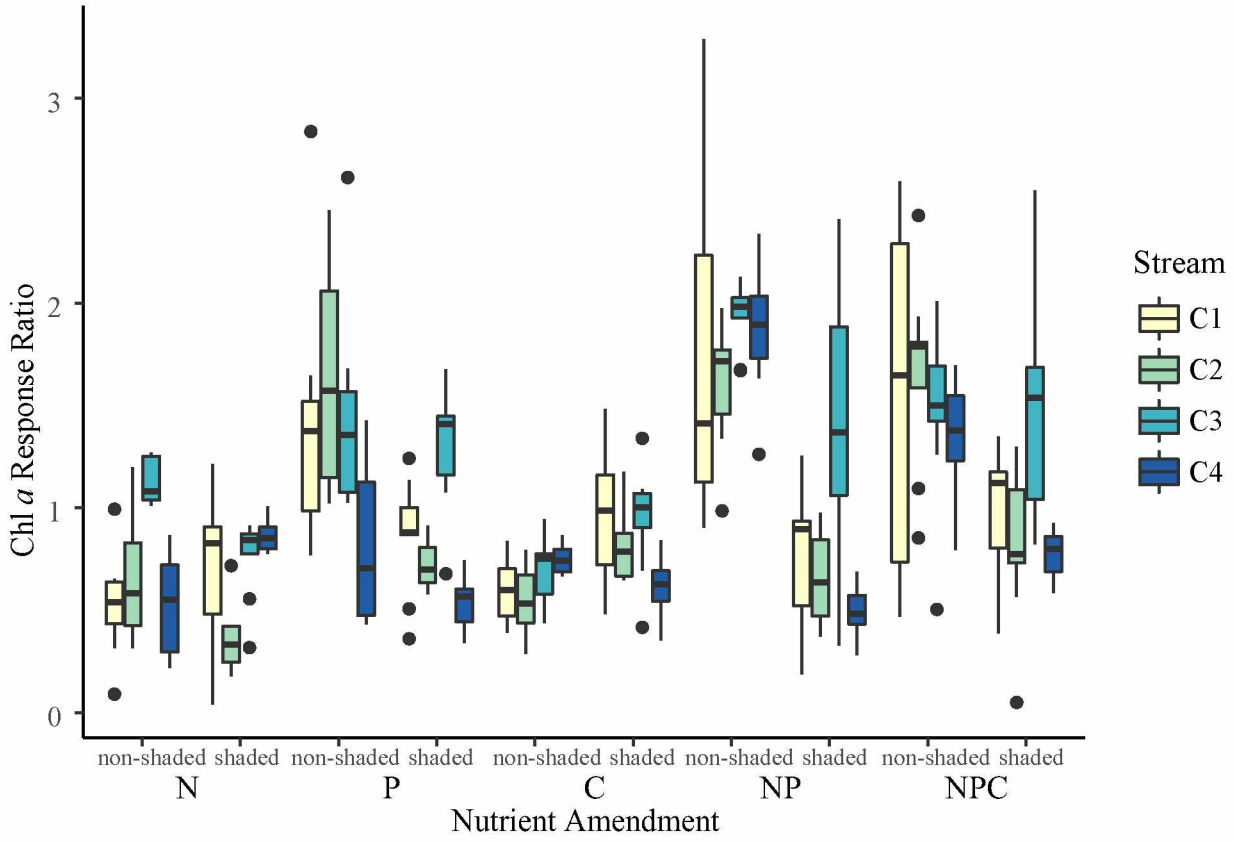

Figure 2.6. Chlorophyll *a* (chl *a*) response ratios (RR) for each study stream and nutrient amendment. Response ratios were calculated by dividing each nutrient amendment (n = 9) by unamended control means. The center lines, box extent, error bars and points indicate the 50th percentile (median), the 25th and 75th percentile (interquartile range), the 95% confidence intervals, and outliers, respectively.

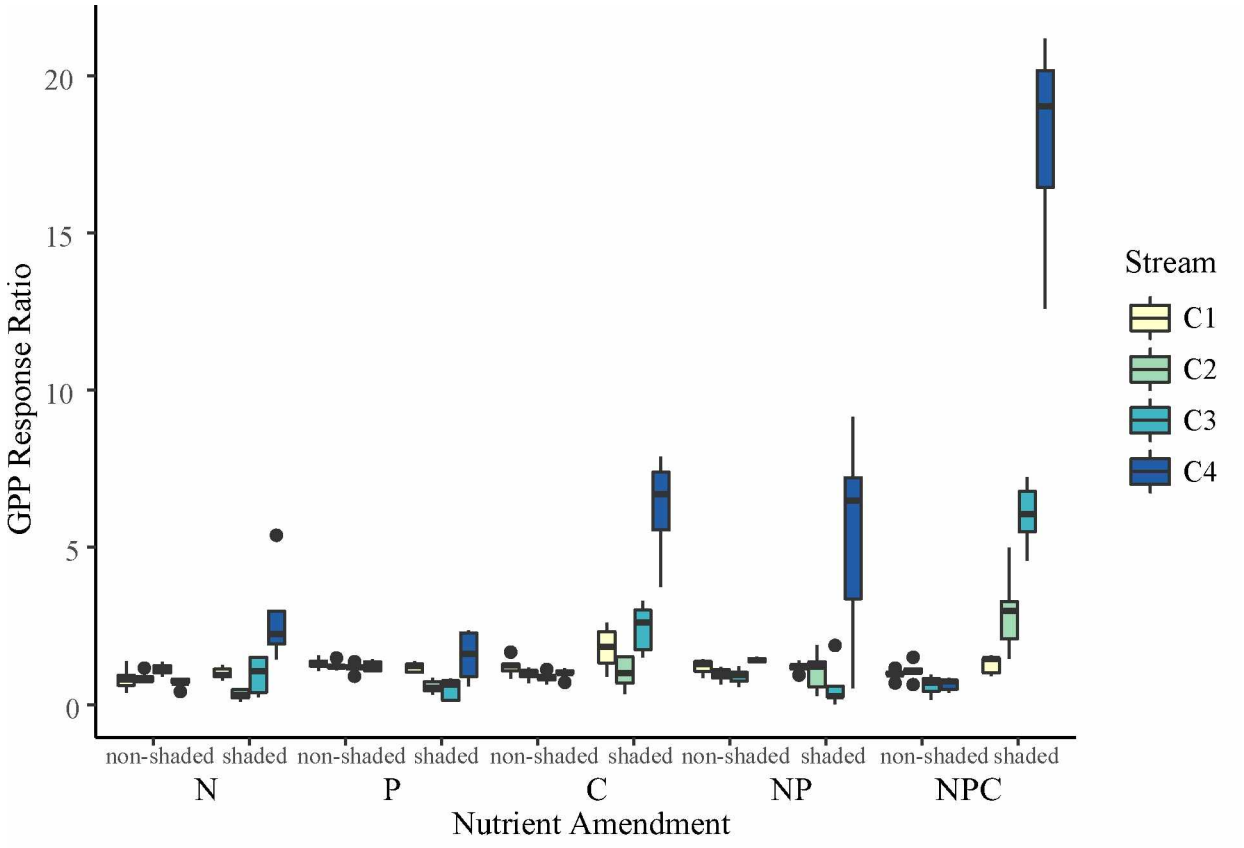

Figure 2.7. Gross Primary Production (GPP) response ratios (RR) for each study stream and nutrient amendment. Both shaded and non-shaded treatments displayed. The center lines, box extent, error bars and points indicate the 50th percentile (median), the 25th and 75th percentile (interquartile range), the 95% confidence intervals, and outliers, respectively.

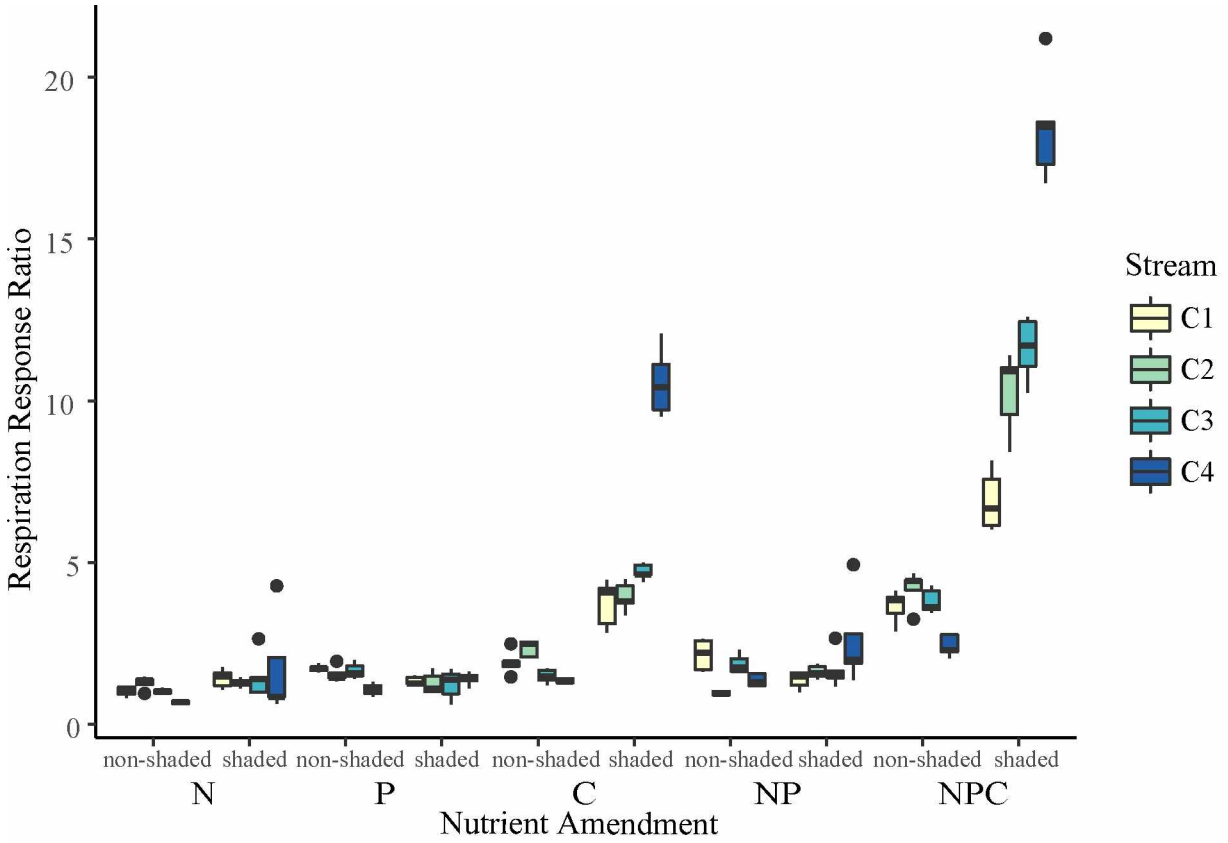

Figure 2.8. Respiration response ratios (RR) for each study stream and nutrient amendment. Response ratios were calculated by dividing each nutrient amendment (n = 5) by unamended control means. The center lines, box extent, error bars and points indicate the 50th percentile (median), the 25th and 75th percentile (interquartile range), the 95% confidence intervals, and outliers, respectively.

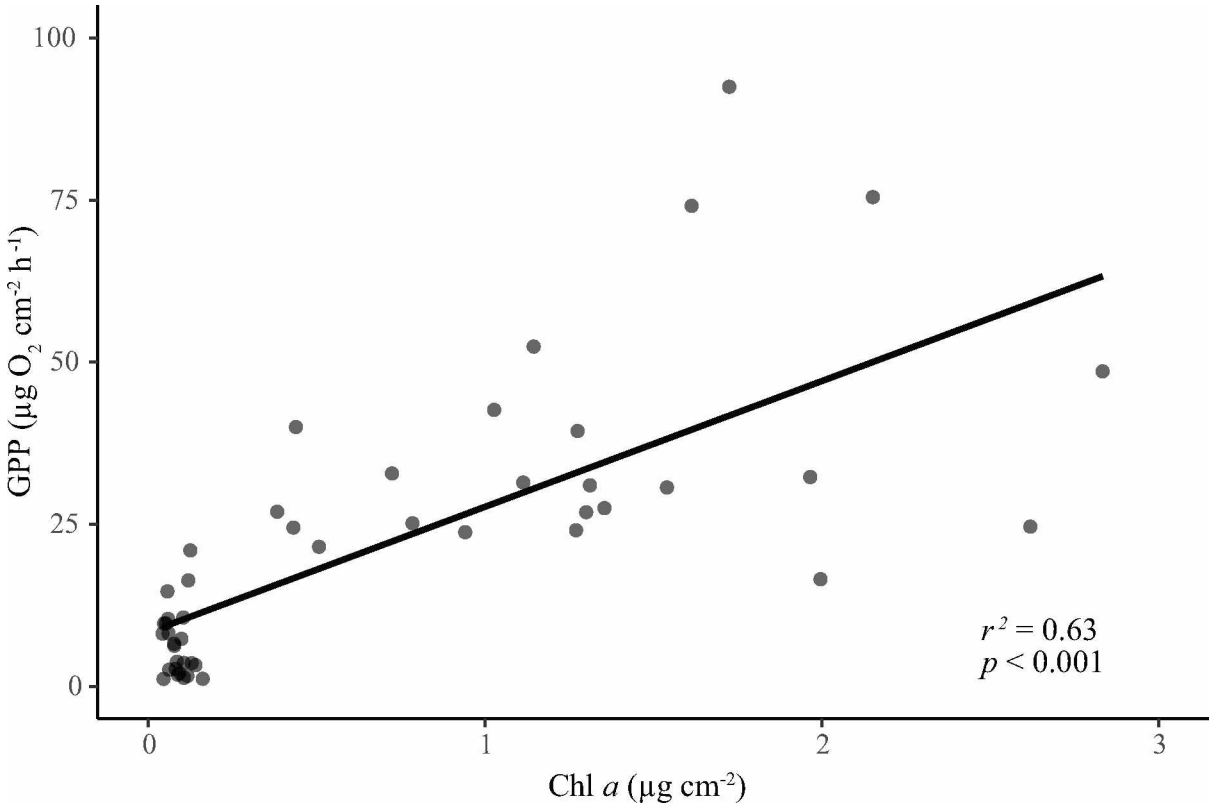

Figure 2.9. Relationship between chlorophyll *a* (chl *a*) and gross primary production (GPP). Plot includes points for all NDS biofilms of all treatments colonized during NDS deployments in all study streams. Points represent treatment means ($n = 240$), including both shaded and non-shaded treatments. The line represents the trend ($r^2 = 0.63$; $p < 0.001$).

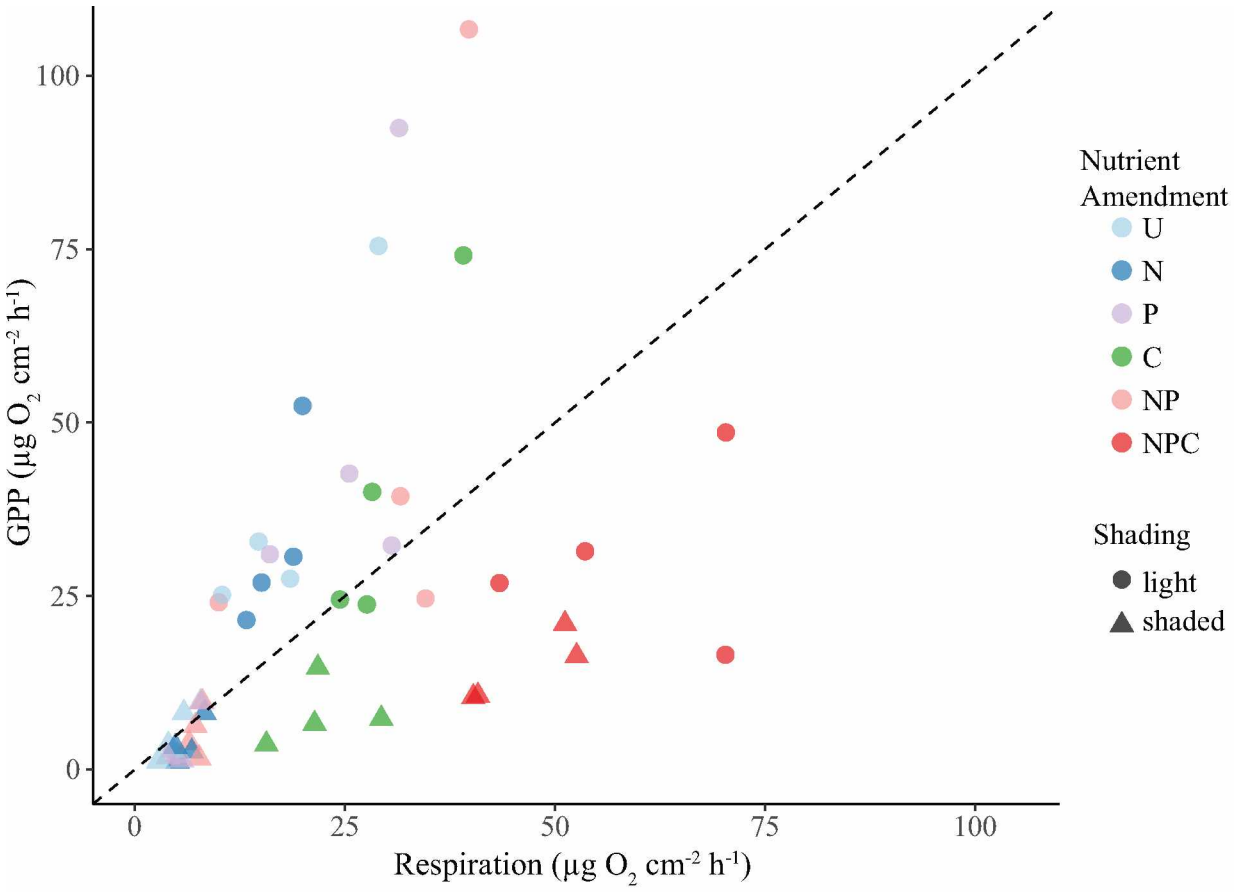

Figure 2.10. Relationship between gross primary production (GPP) and respiration. Plot includes biofilms colonized on NDS fritted glass filter disks across all streams and treatments. Circles represent non-shaded treatment means (n = 5) and triangles represent shaded treatment means (n = 5). Colors represent specific nutrient treatments. 1:1 ratio of GPP to respiration is represented by dashed line.

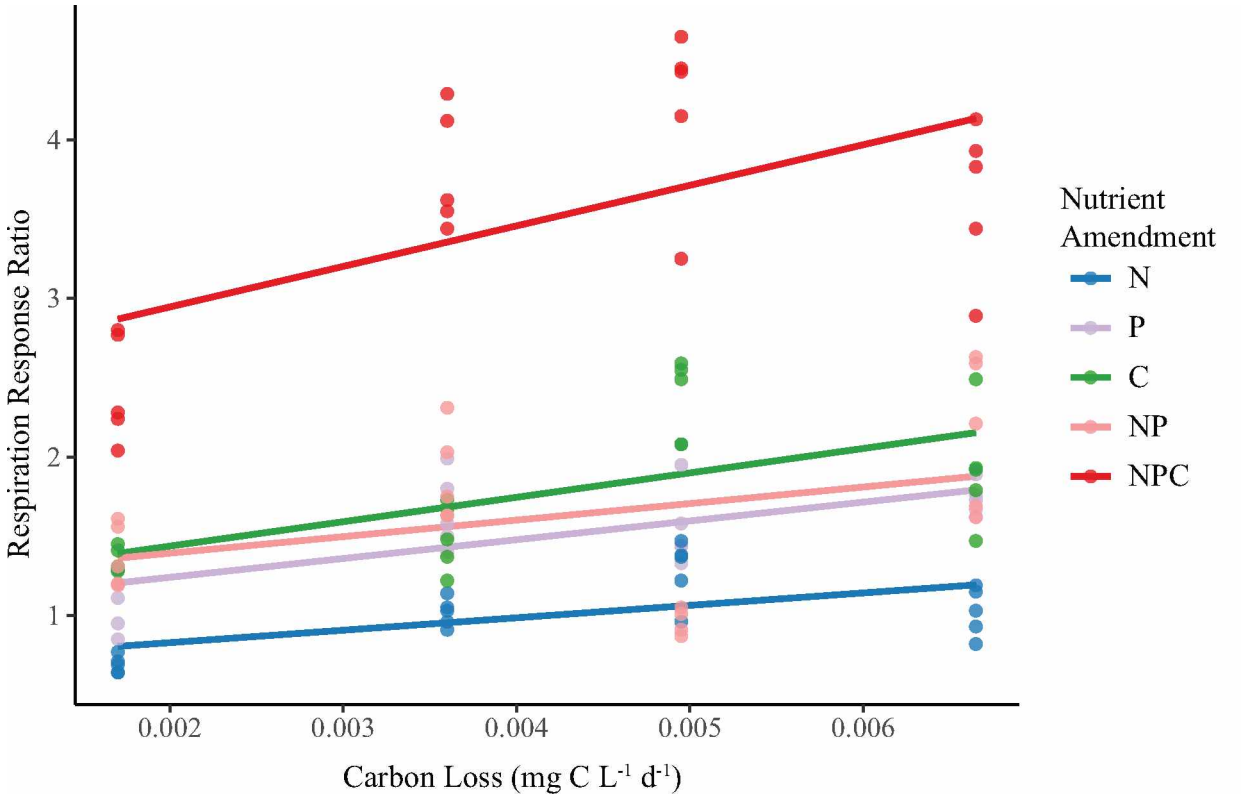

Figure 2.11. Relationship between respiration response ratios (RR) and carbon (C) loss. Plot includes data for each nutrient amendment for non-shaded nutrient diffusing substrata (n = 5) in all streams plotted against mean loss of C (n = 3) over 40-day labile dissolved organic C (LDOC) incubations (simple linear regression: RR_N r^2 = 0.34, p = 0.006; RR_P r^2 =0.48, p < 0.005; RR_C r^2 = 0.38, p = 0.0038; RR_{NP} r^2 = 0.13, p = 0.119; and RR_{NPC} r^2 = 0.36, p = 0.005).

Tables

Table 2.1. Catchment characteristics at the Caribou-Poker Creeks Research Watershed.

Stream	Catchment Area (km^2)	Elevation (m)	Aspect	Permafrost (% cover)
C1	6.7	325	E	26
C2	5.2	323	S	4
C3	5.7	274	NE	53
C4	10.0	226	SSE	19

Table 2.2. Mean ambient water chemistry data from summer 2017 and 2018, and 20-day nutrient diffusing substrata (NDS) incubations. Water chemistry was quantified from autosampler and mainstem water samples.

	Stream	Discharge ($L\ s^{-1}$)	Temp (ºC)	pH	NO_3^- ($\mu g\ N\ L^{-1}$)	NH_4^+ ($\mu g\ N\ L^{-1}$)	DON ($\mu g\ N\ L^{-1}$)	PO_4^{3-} ($\mu g\ P\ L^{-1}$)	TOP ($\mu g\ P\ L^{-1}$)	DOC ($mg\ C\ L^{-1}$)
2018	C1	173.0*	3.62*	6.82	301.5	26.28	75.78	2.51	2.46	2.68
	C2	62.95	4.57	7.12	629.6	24.83	89.19	3.58	2.23	2.14
	C3	75.10	1.97	7.07	561.2	30.52	98.85	3.25	2.18	3.61
	C4	115.3	3.85	7.32	657.6	26.54	79.39	3.02	2.04	1.78
2017	C1	49.21*	2.25*	6.94	328.9	39.42	39.85	2.01	2.35	3.19
	C2	28.21	3.82	7.21	512.6	37.41	49.92	1.58	2.06	2.52
	C3	34.09	2.05	7.07	503.1	53.79	37.07	1.89	2.66	4.09
	C4	57.30	3.83	7.47	627.9	39.96	38.21	2.56	2.49	1.95
NDS	C1	-	4.23*	6.82	260.4	20.10	100.23	2.80	0.58	2.28
	C2	58.71	3.64	7.12	586.4	21.71	70.80	4.04	0.73	1.92
	C3	70.23	1.60	7.09	550.9	23.91	100.42	2.87	1.10	3.28
	C4	105.4	3.29	7.42	658.6	23.99	69.65	3.62	1.13	1.63

*C1 is the only stream where discharge and temperature was not measured continuously using pressure transducers and conductivity loggers throughout the summer field season; stream discharge was manually measured using slugs during August uptake experiments and temperature was recorded during biweekly site visits. 2017 season included to show annual variation in water chemistry at the CPCRW.

Table 2.3. Dissolved organic carbon (C) lability in study streams in 2018. C loss measured as decrease in dissolved organic C (DOC) over 40-day lab incubations.

Stream	Date	DOC ($mg\ L^{-1}$)	C Loss ($mg\ C\ L^{-1}\ d^{-1}$)	C Loss (%)	$SUVA_{254}$ ($L\ mg\ C^{-1}\ m^{-1}$)
C1	6 July 2018	2.38	0.0031	4.75	4.01
	26 July 2018	2.19	0.0102	19.1	3.19
C2	6 July 2018	1.85	0.0071	14.4	3.38
	26 July 2018	1.98	0.0028	7.4	2.38
C3	6 July 2018	3.57	0.0021	2.09	3.59
	26 July 2018	2.98	0.0051	7.3	2.72
C4	6 July 2018	1.66	0.0028	7.8	2.72
	26 July 2018	1.60	0.0006	1.79	2.24

Table 2.4. Nutrient limitation of chlorophyll *a* (chl *a*), gross primary production (GPP), and respiration in study streams. Nutrient limitation of each individual stream was determined by two-way ANOVA with treatment and shading as the two factors. Tukey's HSD post hoc comparisons were used to determine *p*-values. Each nutrient treatment (in non-shaded treatments) was compared to unamended control (U) to determine limiting resource of each stream. Respiration *p*-values for shaded and non-shaded treatments are displayed because non-shaded treatments include both autotrophic and heterotrophic respiration, while shaded treatments include only heterotrophic respiration.

Parameter / Stream	P	N	C	N + P	N + P + C	light	1° limitation	2° limitation
chl *a*								
C1	0.570	0.434	0.660	**0.018**	0.236	**<0.001**	light	N, P
C2	**<0.001**	0.237	**0.036***	**<0.001**	**<0.001**	**<0.001**	light	P
C3	**0.019**	0.993	0.396	**<0.001**	**0.010**	**<0.001**	light	P
C4	0.679	**<0.001***	0.423	**<0.001**	0.091	**<0.001**	light	N, P
GPP								
C1	0.381	0.936	0.806	0.881	0.999	**<0.001**	light	-
C2	0.570	0.967	1.000	0.999	0.999	**<0.001**	light	-
C3	0.883	0.994	0.979	0.998	0.079	**<0.001**	light	-
C4	0.296	**0.021***	1.000	**<0.001**	**0.003***	**<0.001**	light	N, P
Respiration	*shaded*							
C1	0.999	0.998	**<0.001**	0.999	**<0.001**	-	C	N, P
C2	0.999	0.999	**<0.001**	0.966	**<0.001**	-	C	N, P
C3	0.999	0.998	**<0.001**	0.969	**<0.001**	-	C	N, P
C4	0.999	0.999	**<0.001**	0.942	**<0.001**	-	C	N, P
	non-shaded							
C1	**0.007**	1.000	**<0.001**	**<0.001**	**<0.001**	0.053	P, C	N
C2	0.114	0.907	**<0.001**	1.000	**<0.001**	**0.045**	C	N, P
C3	**<0.001**	1.000	**0.014**	**<0.001**	**<0.001**	**<0.001**	P, C	N
C4	0.999	0.143	0.063	**0.036**	**<0.001**	**<0.001**	N, P	C

*Means significantly *lower* than unamended controls.

Table 2.5. Phosphorus uptake parameters in study streams. All nutrient uptake experimentation was completed during August and September 2017 and 2018. Calculations are based on the tracer additions for spiraling curve characterization (TASCC) method and units include: $S_{w\text{-}amb}$ in m; U_{amb} in µg P m^{-2} min^{-1}; and $V_{f\text{-}amb}$ in mm^{-2} min^{-1}. Blank cells represent that uptake experiments were not preformed, while dashes indicate no detectable uptake.

				Stream			
Year	Nutrient Amendment	Shading Effect	Uptake Parameter	C1	C2	C3	C4
2017	+ P	light	$S_{w\text{-}amb}$	417.5	502.36	1419.9	-
			U_{amb}	18.7	13.29	3.06	-
			$V_{f\text{-}amb}$	8.77	5.26	2.19	-
	+ NP	light	$S_{w\text{-}amb}$	646.82	263.05	578.13	453.95
			U_{amb}	18.17	26.72	13.95	34.3
			$V_{f\text{-}amb}$	5.66	10.05	5.38	6.91
2018	+ P	light	$S_{w\text{-}amb}$	343.65	447.46	557.12	
			U_{amb}	42.85	27.6	20.17	
			$V_{f\text{-}amb}$	27.79	12.78	9.95	
	+ P	shaded	$S_{w\text{-}amb}$		-	1085.59	
			U_{amb}		-	13.07	
			$V_{f\text{-}amb}$		-	5.11	
	+ NPC	light	$S_{w\text{-}amb}$		1077.08	554.5	
			U_{amb}		9.74	34.08	
			$V_{f\text{-}amb}$		5.29	10	
	+ NPC	shaded	$S_{w\text{-}amb}$		-	1073.69	
			U_{amb}		-	11.68	
			$V_{f\text{-}amb}$		-	5.17	

Chapter 3: General Conclusions

Stream biofilms consist of diverse assemblages of microbes that perform essential biogeochemical processes and recycle nutrients within stream ecosystems. This research focused on resource limitation of autotrophs and heterotrophs within boreal stream biofilms along a permafrost and water chemistry gradient in sub-arctic Alaska. Through patch-scale nutrient diffusing substrata deployment, we determined that autotrophs were limited by light and inorganic phosphorus (often in combination with inorganic nitrogen), while heterotrophs were primarily limited by a labile carbon source. As carbon lability increased in our study streams, microbial response (as respiration) to nutrient enrichment increased. Reduced autotrophic productivity, and algal suppression by heterotrophs, in the presence of a labile carbon source revealed that heterotrophs likely outcompete autotrophs for inorganic nutrients when they have access to a bioavailable energy source.

Nutrient uptake experimentation at the whole stream scale, however, revealed contrasting patterns to the patch scale. At the reach scale, labile carbon availability did not increase heterotrophic nutrient uptake, yet light positively affected phosphorus uptake. These results suggest that nutrient limitation can differ at the patch and reach scale, due to spatial and temporal variation. While nutrient diffusing substrata allow simple categorization of nutrient limitation by focusing on a small, relatively controlled patch in a stream, whole stream nutrient uptake experiments involve larger stream reaches comprised of complex, heterogeneous habitats encompassing more diverse biological activity (Tromboni et al. 2018). Dissimilar patterns at varying scales reiterate the importance of scaling up from patches to fluvial networks as a whole to more fully understand the biogeochemical processes occurring within a stream.

As a changing climate reshapes nutrient use and retention in boreal forest biofilms, microbial processing in streams will be impacted by increasing temperatures, elevated nutrient exports (Frey et al. 2007), and increased carbon concentration (Reyes and Lougheed 2015). These carbon and nutrient stocks will mobilize into stream networks with permafrost degradation (Balcarczyk et al. 2009), and increased heterotrophic microbial decomposition could lead to increased CO_2 production (Vonk et al. 2015). This increased microbial processing and CO_2 loss from high-latitude streams has the potential to further contribute to permafrost-climate feedbacks.

3.1 Literature Cited

Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry 94:255-270.

Frey, K. E., J. W. McClelland, R. M. Holmes, and L. C. Smith. 2007. Impacts of climate warming and permafrost thaw on the riverine transport of nitrogen and phosphorus to the Kara Sea. Journal of Geophysical Research: Biogeosciences 112:1-10.

Reyes, F. R., and V. L. Lougheed. 2015. Rapid Nutrient Release from Permafrost Thaw in Arctic Aquatic Ecosystems. Arctic, Antarctic, and Alpine Research 47:35-48.

Tromboni, F., S. A. Thomas, B. Gücker, V. Neres-Lima, C. Lourenço-Amorim, T. P. Moulton, E. F. Silva-Junior, R. Feijó-Lima, I. G. Boëchat, and E. Zandonà. 2018. Nutrient Limitation and the Stoichiometry of Nutrient Uptake in a Tropical Rain Forest Stream. Journal of Geophysical Research: Biogeosciences 123:2154-2167.

Vonk, J. E., S. E. Tank, P. J. Mann, R. G. M. Spencer, C. C. Treat, R. G. Striegl, B. W. Abbott, and K. P. Wickland. 2015. Biodegradability of dissolved organic carbon in permafrost soils and aquatic systems: a meta-analysis. Biogeosciences 12:6915-6930.